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THE EARLY STAGES IN THE DEVELOPMENT OF THE HYPOPHYSIS OF AMIA CALVA.

J. M. PRATHER.[1]

THE results of my work on Amia do not agree with the common assumption that the pituitary body is always of epiblastic origin. This disagreement has led me to an examination of the literature on the subject to see if there is sufficient unity of opinion among recent investigators to warrant such a general conclusion. As a result it is found that a diversity of opinion still prevails, and that it is unsafe to predict its origin in any class of vertebrates.

A brief classification of the various views and their respective advocates is of interest in this connection: K. E. von Baer ('**28**), Huschke ('**54**), and F. Schmidt ('**62**) believed the hypophysis to be a modified part of the brain. Reichert ('**40**) and His ('**68**) claimed that it is derived from the end of the chorda. Reichert ('**61**) and Rathke ('**61**) believed it to be derived from the pia mater, each having changed his earlier view. Dursy ('**68**) maintained that it is of threefold origin — from the foregut, the chorda, and the brain.

The above represent the earlier but now generally discarded hypotheses. The more modern views, some of which were also held by the older anatomists, may be grouped as follows:

1. *That the hypophysis is of hypoblastic origin*, as held by

[1] This study was undertaken at the suggestion of Dr. A. C. Eycleshymer and completed under his direction in the Department of Anatomy and Histology in the University of Chicago during the summer of 1899.

Rathke ('38), Luschke ('60), Kölliker ('61), Miklucho-Maclay ('70), W. Müller ('71), His ('75), Hatschek ('81), Dohrn ('81), Owen ('82), Balfour and Parker ('82).

It should be emphasized that, in general, this was claimed for the particular form investigated, but not claimed to hold true for all vertebrates.

2. *That the hypophysis is of epiblastic origin*, as claimed by Goette ('72), Balfour ('74), Mihalkovics ('74), Kölliker ('76), Cattie ('81), Julin ('81), Dohrn ('82), Kraushaar ('83), Johnson and Sheldon ('86), Orr ('87), Scott ('87), Kupffer ('90), Lundborg ('94), Dean ('96), Haller ('96), Braem ('98), Minot ('98).

It should be added that the majority of these observers not only claimed the hypophysis to be of epiblastic origin in the particular form examined, but also believed this to hold good for the Vertebrata.

3. *That the hypophysis is partially of hypoblastic and partially of epiblastic origin*, as positively maintained by Kupffer ('93), Valenti ('95), and Nussbaum ('96), and considered probable by Hoffmann ('85), Orr ('87), and Gaupp ('93).

The researches made by the above-named writers show that the organ varies so much in its development and structure in the different forms that generalizations should be made with extreme caution, until more extended and precise observations have been made.

While its development has been more or less carefully traced in animals representing nearly every group of vertebrates, I find that the Ganoids have received but little attention. Kupffer has described and figured its earliest stages in *Acipenser sturio*. This author finds such an unusual mode of development that it seems possible that he has misinterpreted certain structures connected with the development of the sucking discs, for, as the sequel will show, at a certain stage in their formation in Amia these discs present appearances very similar to those regarded by him as the beginnings of the hypophysis. A fuller discussion is reserved for a later page.

Balfour and Parker have figured some of its early stages in *Lepidosteus osseus*, but have given no adequate description of its origin. Judging from their figures and few

remarks, its early history is in most respects quite similar to that in Amia.

Dean has figured a very early and a very late stage in its formation in *Amia calva*. No description is given of these stages, but the generalizations made therefrom seem, in the light of my researches, to be somewhat questionable, and to them I shall later recur.

Concerning the observations by Professor Minot I know nothing further than the simple statement in *Science*, Feb. 18, 1898, that he had confirmed and extended the results of B. Haller. As Haller's extensive observations did not embrace the Ganoids, I infer that this statement does not pertain to Minot's investigations upon Amia.

With these facts before us it will be seen that a detailed account of the development of the hypophysis in the last mentioned form will not be superfluous.

The sections for this study were placed at my disposal by Dr. Eycleshymer, and consist of series of sagittal, horizontal, and transverse sections of the embryo and larva, in many successive stages of development, up to and including the thirty-fifth day after fertilization. All ages given in the following descriptions are reckoned from the time of fertilization.

Many examinations were made with the oil-immersion and all the drawings have been made with the aid of the camera lucida. The literature consulted, and that to which reference has been made, consists for the most part of papers enumerated by Kupffer[1] and Haller.[2] Since a very complete bibliography is given by each of these authors it would be superfluous to duplicate them in the present paper.

Description of Stages.

The earliest stage in the formation of the hypophysis, clearly recognizable as such, is found in an embryo a few hours before

[1] Kupffer, C. von, "Die Entwicklung des Kopfes von Acipenser Sturio an Medianschnitten untersucht." München und Leipzig. 1893. "Die Deutung des Hirnanhanges," *Sitzungsber. d. Gesell. f. Morph. u. Phys. in München.* Jahrg. 1894.

[2] Haller, B., "Untersuchungen über die Hypophyse und die Infundibularorgane," *Morph. Jahrb.* 1897.

hatching, as shown in Figs. 2 and 3. In order to show the relations of position which the various organs bear to one another prior to the differentiation of the hypophysis, I figure a median sagittal section of an embryo surrounding about 245° of the circumference of the yolk, corresponding to an age of about 148 hours (Fig. 1). At this stage the foregut (*fg.*) is seen to have formed as far back as the posterior limit of the third primary vesicle (*hb.*), where the endoderm forming its wall is reflexed upon itself and passes forwards again over the surface of the yolk (*y.*). By tracing the foregut through the successive sections of the series, we find it to be a very broad cavity compressed dorso-ventrally until its upper and lower walls are in close apposition in the region immediately under the base of the first primary vesicle, which rests directly upon its dorsal wall. Its walls are slightly separated both anterior and posterior to this region. In the median plane, as shown by the figure, the cavity can be traced forwards to a point somewhat anterior to the front wall of the brain (*br.*). From its anterior end a diverticulum may be traced on either side in front of the brain, nearly to the dorsal median plane. These endodermal diverticula later become transformed into the larval adhesive organ, as has recently been shown. The endodermic layer increases in thickness anteriorly until a maximum thickness is attained in the adhesive organ just mentioned. The walls of the endoderm cells cannot be distinguished, owing to the great amount of yolk material found in them.

The ectoderm at the anterior end is invaginated in two places. The upper invagination (*o.*) is merely a depression within the adhesive organ which is developing beneath the ectoderm by diverticula from the foregut, as just described, pushing the ectoderm outwards in the form of a circular ridge (*ao.*) around the snout. The lower invagination is the involution for the stomodaeum (*st.*), and has already pushed inwards nearly to the endoderm surrounding the foregut. This invagination is on a lower plane than the foregut and is directed distinctly downwards, making a small angle with that plane.

The large cavity (*c.*) between the brain and the epiblast in front is for the most part filled by the developing adhesive

organ. But surrounding this may be seen mesodermal cells which form a strand running downwards between the stomodaeum and foregut and connecting with those forming the heart (*h.*) below. The chorda extends no further forwards than the posterior margin of the hind brain. The brain is not as clearly delimited from the ectoderm above as the figure indicates. The first primary vesicle (*fv.*) is evaginated at its base both in front and behind, giving rise to the recessus opticus (*ro.*) and the infundibulum (*in.*) respectively. The base of this vesicle is arched considerably and conforms closely to the dorsal wall of the foregut.

In an embryo about 160 hours old (Fig. 2) marked changes may be noted in all the organs described in the preceding stage. The adhesive organ has broken through the ectoderm, forming a semicircular row of sucking cups on either side of the snout. The section, not being exactly vertical, passes through one of these cups (*sc.*) on the dorsal side. The space between this organ and the brain is now filled with mesoblastic cells (*ms.*). The stomodaeal invagination has deepened a little, owing to the development of the sucking disc over and in front of it. By the vertical growth of the brain the anterior end of the alimentary canal has been pushed downwards to a level with the mouth fold, so that the ectoderm lining the stomodaeum is in close contact with the endoderm forming the wall of the foregut. The oral plate (*op.*) thus formed is on the point of breaking through, and the point of fusion of the endoderm roofing the gut with the ectodermal roof of the stomodaeum is scarcely recognizable. There is no fold of the ectoderm comparable to "Rathke's pocket" discernible, nor is there an endodermal fold comparable to "Seesel's pocket." The ectoderm immediately over the stomodaeum is much thickened and composed of large cells of irregular shape loosely aggregated.

Fig. 3, an enlarged portion of the same section, will show that this ectodermal layer (*ec.*) terminates rather abruptly at the point of junction (*pf.*) with the endoderm (*en.*), at which point the cells are seen suddenly to become smaller. While they are somewhat disconnected in this region, they soon become arranged into three definite layers running back under the ante-

rior part of the thalamencephalon as far as the posterior limit of the optic chiasma (*oc.*). Here, immediately under the central part of the thalamencephalon (*lpo.*), the hypoblastic cells may be seen to have assumed a different shape and size, and the number of layers to have increased. From roundish or ovoid cells they become long, spindle-shaped, much smaller, and arranged in crescent-shaped layers about six in number and fitting one within another, so that the whole mass of cells over a region about 180 μ in length is nested in this peculiar manner. This is the first trace of the hypophysis (*hy.*) recognizable. Back of this differentiation the cells of the endoderm are again of the same rounded or ovoid shape as in front and arranged in two layers. Thus, I repeat, there can be detected no fold or overgrowth of ectoderm to give rise to the hypophysis nor evagination of the endoderm. Its cells are differentiated *in situ* apparently by longitudinal division of the cells constituting the roof of the foregut.

The chorda at this stage extends forwards under the hind brain to near the tip of the infundibular fold, which has greatly enlarged. The base of the thalamencephalon has elongated and is now a single layer of cells (*lpo.*) posterior to the chiasma, resting closely upon the dorsal wall of the foregut.

In a larva early in the eighth day, shortly after hatching, a sagittal section (Fig. 4) shows the oral plate broken in the center. But sections of the series on either side show the membrane or its remnants still intact, stretching across the oral cavity from a point near the tip of the now forming lower jaw to a point (*op.*) on the dorsal side of the cavity just forwards of the anterior limit of the thalamencephalon where it rests upon the dorsal wall of the foregut. It is thus seen that the position of the membrane relative to surrounding parts has not changed from the preceding stage. The floor of the foregut, however, has dropped down in the anterior part, making a rather deep cavity (*fg.*) immediately below the thalamencephalon and behind the oral plate. The dimensions of the thalamencephalon have increased vertically and decreased antero-posteriorly. The infundibular fold has apparently widened, but not deepened. The optic chiasma has greatly thickened, while the base of the

brain remains one cell in thickness and rests flatly upon the dorsal wall of the gut and the hypophysis. The sucking disc (*sc.*) is relatively at its largest size. The cavity between it and the brain is filled with a denser aggregation of mesoblastic cells than in the previous stage.

Fig. 5 shows the base of the brain and the hypophysis of the same section more highly magnified. The hypophysis now measures 150 μ in length and 28 μ in thickness. This apparent shortening may be due to the uncertainty of the limits of the organ in the previous stage, or to individual variations in the two larvae. That peculiar nesting of the cells in the hypophysis, as described above, will be seen to continue, but the cells are now perceptibly larger, while the epithelial cells (*em.*) forming the roof of the mouth beneath the hypophysis have again attained the size and shape of those with which they are continuous in front and behind. The basal layers of endoderm, however, still seem to be dividing, adding new layers to the base of the hypophysis by proliferation. The roof of the mouth is now three layers deep both before and behind the hypophysis.

A transverse section (Fig. 6) of a larva about eight days old, through the posterior part of the hypophysis and the infundibulum, shows that the organ is at this stage convex on the dorsal side in a transverse plane, fitting into a concavity in the base of the infundibulum lying closely upon it. An examination of the successive sections forwards and backwards from this shows that the upward convexity gradually diminishes forwards, but more abruptly backwards. This figure, in connection with the figures of longitudinal sections of larvae both older and younger, shows that the organ is now approximately lenticular in shape. In transverse, as in longitudinal sections, that characteristic nesting of the cells differentiated to form the hypophysis is found to prevail, a basin-shaped stratum of lenticular cells formed from the deeper layers of the endoderm, with other similar strata, each successively smaller, fitting into the previous ones, until we get a nest of seven or eight basins, while the hollow of the upper basin is filled to a rounded fullness with cells of a more nearly uniform diameter, rounded or

polygonal, and with no evident order of arrangement. This fact is strikingly evident, that the limits of the organ, both longitudinally and transversely, mark very accurately the borders of the area over which the brain is in such close contact with the hypoblast.

The mouth cavity posterior to the oral plate is now rather shallow but very wide. Just over the oral epithelium roofing the mouth, on either side of the hypophysis and the infundibulum, the internal carotid arteries (*v.*) are forming, while dorsal to these, in the folds formed between the infundibular lobe and the thalamocoele above, may be seen two other large cavities (*v'*), the cavities of the preoral somites.

A sagittal section of a larva about nine days old (Fig. 7) shows the base of the thalamencephalon (*fv.*) to be a single layer of columnar cells where it rests on the hypophysis, but thickened in front for the chiasma and likewise behind in the infundibular region, which has grown larger. The end of the chorda approaches very near to the infundibulum, but remains separated therefrom by mesoblastic cells, which may be seen to grow in between the brain and mouth roof to the limits of the hypophysis at either end. The lower jaw is relatively further back, its tip now lying under the anterior end of the hypophysis. The epithelium of the mouth is continuous beneath the hypophysis, but the basal layer of endoderm appears to continue to divide, adding new cells to the hypophysis by proliferation. The marked difference between the shape and arrangement of the cells in the upper and lower portions of the organ may be noticed still. At the posterior end the body is distinctly separated from the mother layer, while at the anterior end its cells take on more and more the character of the mother layer as we go forwards. The organ measures in this plane 196 μ in length by 41 μ in thickness.

A transverse section of a slightly older stage (Fig. 8), during the tenth day, shows an oval-shaped organ 130 μ in breadth by 64 μ in thickness. It is clearly separated from the endoderm at either side, so that it seems to be wholly differentiated, but still lying in a depression in the endoderm caused by the transformation of the cells of this layer into cells of the hypophysis.

The characteristic stratification on the ventral side and the irregular grouping of the cells above are still pronounced. Very nearly in the center, between these lower strata and the cells above, is a nearly spherical cavity (*l.*) about 16 μ in diameter, the first lumen that has been recognized. This lumen is not a longitudinal slit, as it is found only in this section; and since it is enveloped by no distinct membrane, and the cells have no definite arrangement about it, it has the appearance of an intercellular space. The mesoderm (*ms.*) is seen to grow between the brain and the mouth up to the sides of the hypophysis, just as at the ends. It would thus appear to be a biconvex body fitting into concavities in the brain above and the endoderm below.

In a larva ten days old, of which Fig. 9 is a sagittal section of the hypophysis and parts adjacent and Fig. 10 a transverse section more highly magnified, the organ has become entirely separated from the mouth roof, which has again become two cells in thickness beneath it. A strand of mesoderm (*ms.*) passes between the hypophysis and mouth roof and is continuous at the sides with the thickened mesoderm differentiating to form the cranial cartilages. An outfold of the infundibular wall at its posterior lower margin, 33 μ by 39 μ internal measurement, is the first stage in the formation of the infundibular gland, or saccus vasculosus (*sv.*). The single layer of cells characterizing the base of the infundibulum and the base of the thalamencephalon is continued into the saccus, but here the cells are columnar, with nuclei at their outer ends, in marked contrast with the cells in the brain wall contiguous. The chorda (*ch.*) runs forwards under the hind brain almost in contact with the roof of the mouth, and its cephalic end (*chh.*) abuts against this infundibular process with a strand of mesoderm between. This mesoderm grows into the space about the hypophysis, and a thin strand of it runs between the organ and the mouth roof below.

The hypophysis now measures in longitudinal section 143 μ by 57 μ, in transverse 136 μ by 66 μ. A lumen may be seen in the center, — an oval cavity with a membrane surrounding. About this lumen the spindle-shaped cells seem to be arranged in a radiate fashion, with long axes pointing towards it.

In a larva about fourteen days old a median sagittal section (Fig. 11) shows marked changes in surrounding parts, with but little change in the hypophysis. The saccus vasculosus has grown until it measures at this stage 120 μ in length, and from 6 μ in width at its point of origin to 27 μ at its widest part. Its tip now touches the base of the hind brain. The columnar cells which characterized it in the preceding stage are yet very noticeable, the transition to the rounder cells of the infundibular wall above being very abrupt, while the transition to the cells of the infundibular base is more gradual. Beneath, the hypophysis rests directly upon a strand of fibrous tissue (*ms.*) continuous before and behind with the perichondrium surrounding the sphenoidal cartilages, which are encroaching from all sides. The membrane roofing the mouth (*em.*) has become widely separated from the hypophysis, and in it dental protuberances and glandular cells have differentiated. The hypophysis has now attained a size of 156 μ by 56 μ, and has several small spherical or lenticular cavities which do not communicate.

A sagittal section of the hypophysis of a larva about the same age is shown in Fig. 12 strongly magnified. It will be seen that a few mesodermal cells have come to lie between the hypophysis and the brain, forming a thin layer separating the two organs. With this interpolation of mesoblastic tissue the lobing of the hypophysis begins, and this continues to increase with age. A large central oval lumen is conspicuous at every stage, while in the section here figured, several smaller lumina are met with as one examines the sections of the series. The principal lumen in this case measures 19 μ by 6 μ, and can be detected in but two sections, showing that its shape is lenticular. The smaller ones are more nearly spherical, and each is found in but a single section. No communication between them can be found. The hypophysis in this larva measures 164 μ in length by 57 μ in thickness. The characteristic radiate arrangement of the cells about the lumina is very noticeable.

Fig. 13 shows the relations of the hypophysis to surrounding parts, at a stage about one day older, in a transverse section through the middle of the organ. The oral epithelium has

further differentiated. The lateral cartilages (*sk.*) of the skull are closely encroaching from the sides, enclosing the internal carotids (*bv*). These cartilages are connected by a strand of the perichondrial membrane which runs beneath the hypophysis. The organ is almost perfectly lenticular in cross-section, measuring 180 μ by 70 μ. A principal lumen is seen in the center, but others are found at other positions in sections in front and back of this one.

In a larva between twenty-two and twenty-six days old, a cross-section (Fig. 14) shows the hypophysis appreciably enlarged, measuring now 220 μ by 75 μ. An irregular but distinct lobing may be seen, more pronounced on the upper than on the lower side. In this way, from a very symmetrical organ in the fifteen-days stage, we get here a marked asymmetry. It is here firmly adherent to the infundibulum, but quite apart from the wall of the cranial cavity. This separation from the base of the cranial cavity may be considered an artificial condition, as succeeding stages invariably show it to be in contact with this wall. The distinct upward bend in the infundibular base, the enlargement and gradual encroachment of the lateral cartilages, and the advancing differentiation of organs in the oral epithelium may be remarked.

The same section of the hypophysis highly magnified (Fig. 15) shows its histological structure to present some interesting features. The large central lumen with its very definite lining membrane is a striking object. It is ovoid, measuring 24 μ by 14 μ. Other spheroidal but smaller lumina are to be found near the tip of the different lobes as they are traced by sections, but communication of these lumina with each other, or with the central lumen, cannot be definitely proved. It seems that communication may be had through very narrow channels representing connected intercellular spaces between rather definite rows of cells and running from the lumina of the lobes towards the central lumen. But apparently these channels are closed before reaching the lumen. Such spaces may be seen in the figure (*sl.*) running out into the lobes to the right and to the left. It will be noticed that the cells maintain a rather definite and orderly arrangement about the central lumen. There is a

row encircling the lumen in a radiate manner, long spindle-shaped in the upper and lateral parts, more rounded below. The other cells appear to have a general tendency to arrange themselves in rows pointing towards the center of the organ, but this arrangement is modified by a secondary tendency to be grouped about the secondary lumina and the longitudinal channels. A marked feature of this and succeeding stages is the indistinctness or total obscurity of the nuclei.

A sagittal section at this stage shows the same peculiar arrangement of cells about the lumina and channels in the separate lobes. And the other characters of the hypophysis are similar to those shown in the cross-section. The anterior sphenoidal cartilage has now advanced to the posterior part of the chiasma nearing the hypophysis, the posterior to a point not far from the posterior point of the saccus, while dense skeletogenous tissue continues to and below the saccus nearly to the hypophysis. The perichondrium, as before, stretches across from one cartilage to the other below the organ. The basilar artery is now well formed, running along the base of the hind brain up into the fold between the hind brain and the primary forebrain. A blood vessel may be seen also just posterior to the hypophysis beneath the point of origin of the saccus. The saccus has enlarged, and in its cavity may be seen abundant granular secretions.

Passing from this stage to a stage between thirty and thirty-five days, a sagittal section (Fig. 16) shows all parts much enlarged. The finger-shaped saccus measures internally 311 μ by 18 μ at its narrow opening into the infundibulum, and 77 μ at its widest part. The granular secretions noticed in the previous stage have increased in amount. While the sphenoidal cartilages have enlarged and strengthened compared with the condition in the previous stage, they have approached very little nearer to the hypophysis; but the connective tissue between the hypophysis and the roof of the mouth has considerably increased. The hypophysis at this stage has attained a size of 359 μ by 96 μ. Increased lobing is not apparent from the figure, but the series shows a great increase in the number of lobes and of the lumina in them. The arrangement and char-

acteristics of the cells are not enough different from those described in the preceding stage to call for special remark.

A comparison of sagittal with transverse sections (Fig. 17) demonstrates that in shape the organ retains the general lenticular form acquired early in its formation. It measures now 284 μ in breadth by 88 μ in thickness. The lateral cartilages (*sk.*) have advanced far in towards the hypophysis, so that it may be clearly seen that the organ lies in a distinctive space surrounded by cartilages on all sides — the pituitary fossa. Small bits of connective tissue may be seen between the hypophysis and the brain in the folds of the former, and also in the recesses between the lobes on the under side. The infundibular base is folded more or less in conformity to the lobing of the hypophysis. No evident communication between the cavities nor ducts opening to the exterior have been observed at this, the latest stage studied.

A horizontal section of the hypophysis (Fig. 18) of a larva about 20 mm. in length, thirty days old, shows that the lobing of the organ is principally around the edge ; that its general shape is circular in this plane, lenticular as a solid ; that a cavity may be found near the end of each well-formed lobe, which may possibly communicate with the central lumen by a very narrow indistinctly defined channel ; that the cells are, in general, arranged in a double row around each lobe, the space between the rows constituting the channel mentioned ; that the cells are arranged radially about the lumina. The section figured is not exactly in a horizontal plane, but dips a little posteriorly and to the right, so that the lobes mostly appear to be on the anterior side, but are in reality of approximately the same number in each half. The organ is here seen to be enclosed in the sella turcica, which is far advanced in its formation. The infundibulum fits closely upon its upper surface, the projections of the one fitting roughly into the depressions of the other. No blood vessels can at this stage be seen entering the organ, nor nervous tissue be found connecting it with the brain. It seems not to have become glandular as yet.

Summary.

In distinction from an epiblastic origin, as found in most forms, my observations lead me to believe that the hypophysis is of hypoblastic origin in Amia.

Prior to the differentiation of the hypophysis the foregut extends far forwards ; by diverticula the hypoblast reaches even the dorsal side of the head in front of the brain. At this time the stomodaeal involution is below the front end of the foregut. The diverticula later sever their connection with the foregut and are metamorphosed into the larval adhesive organ. The hypoblast unites with the epiblast on the last day before hatching, seventh day, forming the oral plate at a point forwards of the anterior limit of the brain.

There is no indication of an overgrowth of epiblast between the brain and the foregut, nor is there an invagination from the stomodaeum to give rise to the pituitary body. Neither does an outfold from the hypoblast occur to form it. Its first stage is found near the close of the embryonic period, about 160 hours, as a local differentiation of hypoblastic cells in the dorsal wall of the mesenteron, immediately under the thalamencephalon where the base of the brain is in close contact with the hypoblast. The intimate fusion of the base of the first primary vesicle with the hypoblast, from a time long before the stomodaeum has united with the foregut until after the hypophysis has become well differentiated, seems to me to preclude the possibility of any epiblastic tissue entering into its composition, and leads me to think that its origin is probably due to a mechanical cause. This region of fusion is far back of the oral plate, which remains intact for several hours after the differentiation of the hypophysis has begun.

The growth of the hypophysis is at first apparently due more to the enlargement of the cells first differentiated to form it than to the addition or multiplication of cells. It appears to remain in genetic connection with the mother layer until about the tenth day, when it becomes wholly delimited therefrom by an ingrowth of mesoblastic tissue between it and the mouth roof.

During this early period there is a distinct stratification in the arrangement of cells in its lower portion, as if formed in successive strata by proliferation from the mother layer. This stratification is not apparent in the upper part of the organ, and is no longer seen in the lower part after the ninth day.

The first lumen is formed about the ninth day, from which time on an increasing number of lumina is to be found as the organ develops. A lumen appears near the end of each lobe. These lumina are oval or spherical cavities, and definite channels of communication have not been observed, though indications that such channels are forming are found in the arrangement of the cells about the longitudinal axes of the lobes. The cells have a tendency to arrange themselves radially about the lumina.

The organ is almost perfectly lens-shaped until about the fifteenth day, when the formation of lobes begins with the interpolation of mesoblastic cells between the hypophysis and the brain. The number of lobes multiplies from this time on till the thirty-fifth day, beyond which stage observations have not been made. The lobes form chiefly around the periphery of the lenticular body.

The organ is thus, at this stage, a spongy body with isolated cavities, rather than a complex of glandular tubules which so frequently characterize it. The nuclei of the cells become indistinct or wholly disappear by the twenty-second day. This may indicate a glandular modification, but no evident glandular secretions have been detected. No duct nor external opening of cavities has been observed.

No arteries or blood vessels are to be found in it at the latest stage examined. Neither have nerve fibers been seen connecting it with the brain.

The cranial cartilages developing from the mesoblast increase in size and strength from about the tenth day, until at the thirty-fifth day they closely surround the organ on all sides, forming the pituitary fossa, in which the organ lies in close contact with the infundibulum on the dorsal side, but separated from the mouth by a fibrous strand of connective tissue on the ventral.

The saccus vasculosus begins to form about the tenth day and steadily enlarges, until at the thirty-fifth day it forms a process of the infundibulum in the shape of a glove-finger, extending directly backwards under the base of the medulla and parallel with the base of the cranium.

This is very nearly its position in the adult brain as figured by Allis.[1] At no stage, therefore, is it in close association with the hypophysis, which connection is found to be true in so many cases. Abundant granular secretions are found in it at the twenty-second-day stage, and these increase in amount thereafter.

General Remarks.

The course of development of the hypophysis in Amia as described in the preceding pages, when compared with the descriptions given by other observers, both in Amia and other Ganoids, will be seen to present some striking peculiarities.

Dean[2] says: "The hypophysis is by no means as important an element in the development of the head in Amia as in other Ganoids. Its appearance is late and inconspicuous. It has not been found in stages earlier than that of Fig. O (*hatching time*),[3] and even here its presence is not definite. At the most the position of its lumen can be recognized as the line *HY*, formed by the arrangement of cells immediately below the region of the recessus opticus. These cells are apparently ectodermal, for they are arranged in a continuous line with the cells of the formative epiblast of the dorsal wall of the stomodaeum, but, on the other hand, their ventral limit cannot be distinguished from the entodermal cells roofing the foregut."

In what respect the development of the hypophysis is less important in Amia than in other Ganoids is not clear. In point of size and position its relations are almost exactly the same as in the other Ganoids, so far as the larval stages have been examined. As for its appearance being "late and incon-

[1] Allis, Edward Phelps, "The Cranial Muscles and Cranial and First Spinal Nerves in Amia Calva," *Journ. of Morph.* Vol. xii, No. 3, Pl. XXXVIII. 1897.

[2] "On the Larval Development of Amia Calva," *Zool. Jahrb.*, p. 667. 1896.

[3] Italics are mine.

spicuous," I may say that it arises, as will be shown, at a time intermediate between the time of its first occurrence in the other two forms in which this point has been determined, and it is certainly a conspicuous, I might say, striking differentiation of cells, even in its fundaments, although it may not be a prominent object in point of size or in the distinctness with which its limits may be fixed. The actual time of its appearance I have found to be several hours earlier than the time assigned by Dr. Dean, as a comparison of Fig. 4 with his Fig. O plainly proves. His figure and description indicate a stage nearly the same as my Fig. 4, which is shortly after the time of hatching. Although in his figure the oral plate is still unbroken, the cavity of the foregut posterior to it has noticeably deepened by the dropping downwards of its basal wall, while in the stage I figure the cavity is of about the same depth, and the oral plate is only severed at its middle point, remaining intact at the sides. While in Fig. 4 the cells, which he would call the beginning of the hypophysis, may be said to be "continuous with the cells of the formative epiblast," being in the same plane with them, there is no other evidence of a connection and certainly no ground whatever for considering them derived therefrom, as the enlargement of this section (Fig. 5) makes very clear their differentiation from cells of the hypoblast. But its earliest stage is found (Figs. 2 and 3) some hours previous to this stage, far back of the oral plate and the epiblast, as a well-defined modification of cells of the basal layer of hypoblast where the post-optical lamina of the brain rests closely upon it. Considering the common assumption of embryologists that its origin is from the epiblast, my observations become of interest, since I believe that I have proved beyond question that the hypophysis is of hypoblastic origin, and I do not at present understand how Dean could have considered it otherwise.

Balfour and Parker[1] state in a footnote: "We have not been able to work out the early development of the hypophysis as satisfactorily as we could have wished. . . . Were it not for

[1] "On the Structure and Development of Lepidosteus," *Trans. Roy. Soc.* Pt. ii, p. 379. 1882.

the evidence in other types of its being derived from the epiblast, we should be inclined to regard it as hypoblastic in origin." They speak of it as an invagination of the oral epithelium, without stating whether this invagination is anterior or posterior to the oral plate. Presumably from the previous statement they consider it derived from the roof of the foregut posterior to the oral plate. They figure a transverse section of the anterior part of the head of an embryo on the ninth day after impregnation, showing an invagination from the mouth roof with a thickened, solid, conical process extending upwards into the cranial cavity. This, with the exception of the invagination, is very similar to its condition in cross-sections of Amia late in the seventh day, passing through the anterior end of the organ, as shown in Fig. 6. They also figure transverse sections through the anterior and posterior ends of the hypophysis of an embryo eleven days old, where in front it is still in connection with the oral epithelium, while behind it is constricted from that layer. These sections indicate relations almost exactly the same as transections of the organ in Amia shown in sagittal section (Fig. 7) at an age of not quite nine days. Comparing these few sections of Lepidosteus and the few words of description with the conditions which I find in Amia, it seems that the hypophysis undergoes a nearly parallel series of changes in the two forms from the time of its primary origin to a late larval stage, but that corresponding embryonic stages are met with in Amia from one and a half to three days earlier than in Lepidosteus, and the corresponding larval stages are found earlier and earlier as the animal advances in age. This justifies, so far as the hypophysis is concerned, Dean's statement that "the organogeny of Amia progresses more rapidly than in Lepidosteus."

I find no lumen earlier than the ninth day, when it appears as a single nearly spherical cavity near the center of the organ, rather than a longitudinal slit, as indicated by Dean in Fig. O. Furthermore, as my sections show, the lumen is never a longitudinal slit, even at the late stage of thirty-five days, corresponding to Dean's Fig. Q of a four-weeks-old larva which shows it as such. Instead of a single lumen at this late period,

I find several lumina which apparently do not communicate. Neither do my observations agree with those of Balfour and Parker on Lepidosteus at a corresponding age, where the hypophysis is described as "small, not divided into lobes, and provided with a very small lumen"; whereas in Amia at this period it is much lobed and possesses numerous spherical lumina.

Kupffer (*loc. cit.* p. 59) has described a very early and unusual formation of the hypophysis in *Acipenser sturio*. According to him the organ arises during the second day after fertilization by an invagination of the basal layer of the ectoderm on the dorsal side of the head, in front of the brain, between the yet unclosed neuropore and the sucking disc. This invagination grows downwards and backwards until it comes in contact with the endoderm, and then, before the close of the second day (forty-five hours), by a rupture of this layer, it communicates with the alimentary canal, which communication persists for about twenty-four hours. But before the stomodaeum has formed a connection with the foregut, this union of the hypophysis with the latter has broken off (*i.e.*, by the sixty-fourth hour), and the lower blind end of the tube becomes swollen into a hollow bulb. This bulb gradually enlarges and migrates backwards to a position between the dorsal wall of the gut and the base of the thalamencephalon, while the remaining part of the tube, namely, the stalk, gradually atrophies and has wholly disappeared by the time the embryo is hatched (eighty-seven hours).

Haller has expressed a doubt as to Kupffer's interpretations. This doubt is based upon the indistinctness of the parts observed at this early time. From Kupffer's own words it would seem that the cells were greatly obscured by food-yolk. I give the passage quoted by Haller: "Die Entodermzellen sind noch mit Dotter überladen, die Zellen der Epidermis und des Hirnes schon fast dotterfrei, aber diejenigen Ektodermzellen, die in der Bildung der gleich zu besprechenden Organe (*Hypophysenanlage*, etc., Haller) eingehen, zeigen noch denselben Dottervorrath wie die Elemente des Entoderms und sind daher durch Färbungen von diesen nicht zu unterscheiden. Die Abgren-

zung der einzelnen Theile dieser Region gelang mir erst unter vergleichender Prüfung nahe auf einander folgender älterer Stadien."

I likewise believe that Kupffer has misinterpreted what he saw, but for an entirely different reason from that given by Haller, yet based upon the same passage.

Miss Phelps[1] has recently shown that the "larval adhesive organ" in Amia develops at about the time that Kupffer assigns to the formation of the hypophysis in Acipenser. The adhesive organ begins as a diverticulum from the endoderm anterior to the brain, which later becomes divided into a pair of diverticula whose connection with the endoderm becomes broken off after a time. Acipenser and Lepidosteus each possesses a sucking disc, or adhesive organ, similar to that in Amia and at a corresponding developmental period. It would therefore seem that they should justly be regarded as homologous organs, and, further, we should naturally expect them to have a similar ontogeny. Previous to the observations of Miss Phelps this organ has been assumed to be of ectodermic origin (Dean for Amia, Balfour and Parker for Lepidosteus, and Kupffer for Acipenser).

In my own studies on Amia, as described on a preceding page, I observed these diverticula connecting the foregut with lateral masses of cells lying on either side of the snout and between the anterior end of the brain and the overlying epidermis, filling nearly the whole of this pre-cerebral region. These masses of cells present an appearance exactly like the cells walling the foregut, being laden with much yolk just as Kupffer describes, and could not be distinguished from them. The narrow channels can be traced from the cavity of the foregut upwards through the masses to near the epidermis on the dorsal side where they end blindly. The cells of the epidermis and brain contiguous to these present a very different appearance, due to their freedom from yolk.

I was at a loss to account for these canals connecting the enteric cavity with organs on the dorsal side of the head, until

[1] "On the Development of the Larval Adhesive Organ in Amia." Abstract in *Science*. March 10, 1899.

I saw the account of the development of the adhesive organ by Miss Phelps. I at once concluded that I had not only confirmed her discovery, but had also found wherein Kupffer has probably erred in his interpretation of the structures which he believed to be connected with the development of the hypophysis, but which to my mind have an entirely different meaning. The statement by Kupffer, that the cells overladen with yolk going to form the hypophysis are not to be distinguished from the elements of the endoderm, receives its explanation in the fact that they are endodermal cells growing out from the foregut by evagination. The canal which, according to Kupffer, connects the foregut with the dorsal ectoderm is nothing else, in the opinion of the writer, than this median diverticulum from the foregut, or possibly one of the lateral diverticula resulting from it, running up through the adhesive organ to the point of closure of the neuropore. A slightly oblique dorso-ventral section through this region in Amia during the sixth day of development would show strikingly similar appearances to those figured and described by Kupffer in Acipenser.

As a phylogenetic interpretation of the singular development of the hypophysis in Acipenser, Kupffer holds that we here have traces of the ancestral mouth which opened above the sucking disc and in front of the brain. Its origin from the stomodaeal roof as found in most vertebrates, he claims, is a secondary condition due to a migration downwards from its original position, compelled by the great development of the fore brain in these forms and the concomitant degeneration of the adhesive discs, whose remnants, he thinks, are still to be found in the fold between "Rathke's pocket" and the oral plate.

This explanation fails to account for its origin in Amia and Lepidosteus, in which the sucking disc is still large and functional, and whose fore brain is little different from that found in Acipenser; and yet in them the hypophysis arises at a point far back of the sucking disc and under the brain.

It may be considered highly probable that the hypophysis is

endodermic in origin in Lepidosteus as well as in Amia, and I venture the prediction that a further study of Acipenser will demonstrate the same for that form. The foregut extends far forwards in each of the Ganoids in which its development has been studied, and the writer believes that in all these it arises from the endoderm. A study of the figures of the head parts in those animals in which the hypophysis is undoubtedly of ectodermal origin shows that in them the foregut stops short of the infundibulum, while in some, at least, of those forms in which its origin is questionable — ectodermic or endodermic — the foregut and stomodaeum meet at an intermediate point, directly under the base of the thalamencephalon. These facts lead me to suggest that mechanical factors, acting at the point of fusion of the brain base to the oral roof, may play an important part in determining its development from this or that layer.

If my observations be confirmed, that the hypophysis in Amia is derived from the hypoblast, will this fact strengthen Kupffer's hypothesis that it represents a degenerated "paleostome," or will it rather revive the old theory of Dohrn, that it represents a portion of a canal connecting the alimentary tract with an exterior dorsal opening through the thalamencephalon and the epiphysis and accordingly believed to be homologous with the invertebrate pharynx? I find nothing in its structure or its relations, other than its point of origin, which can be interpreted as evidence in support of either hypothesis. I have given what I believe to be the facts, but must leave their interpretation to the maturer judgment that will come from the more complete and extended observations of the future.

Hull Anatomical Laboratory,
September 22, 1899.

ABBREVIATIONS.

A. anterior.
ao. outfold of ectoderm caused by the developing adhesive organ.
br. wall of brain.
bv. blood vessels.
c. cavity occupied by adhesive organ in its young stages.
ch. chorda.
chh. cephalic end of the chorda.
ec. ectoderm.
egm. egg membrane.
em. epithelium of mouth.
en. endoderm.
ep. epiphysis.
fg. foregut.
fv. first primary vesicle of brain.
h. position of heart.
hb. third primary vesicle of brain.
hy. hypophysis.
in. infundibulum.
l. lumen of hypophysis.
lpo. lamina postoptica.
ls. crystalline lens.
m. mouth cavity.
mb. mesencephalon.
ms. mesoderm.
o. involution of ectoderm between the sucking discs.
oc. optic chiasma.
op. oral plate.
ov. optic vesicle.
P. posterior.
pf. point of fusion of ectoderm with endoderm.
ro. recessus opticus.
s. strand of fibrous tissue continuous with the perichondrium.
sc. sucking cup.
sk. skeletal cartilages.
sl. cleft between rows of cells.
st. stomodaeum.
sv. saccus vasculosus or infundibular gland.
v. internal carotid artery.
v'. head cavities.
y. yolk.

EXPLANATION OF FIGURES.

FIG. 1. Median sagittal section of the anterior portion of an embryo surrounding about 245° of the egg's circumference. About 148 hours. × 60.

FIG. 2. Median sagittal section of the anterior portion of an embryo a few hours before hatching time. About 160 hours. × 60.

FIG. 3. The base of the thalamencephalon, the foregut, and the stomodaeum of the same section under stronger magnification. × 190.

FIG. 4. Median sagittal section through the anterior portion of a larva soon after hatching, early in the eighth day. × 60.

FIG. 5. The roof of the mouth and the hypophysis of the same section more highly magnified. × 190.

FIG. 6. Transverse section through the brain and hypophysis of a larva about eight days old. × 60.

FIG. 7. Median sagittal section through the hypophysis and base of the thalamencephalon of a larva nearly nine days old. × 190.

FIG. 8. Transverse section through the hypophysis and base of the infundibulum of a larva during the tenth day. × 190.

FIG. 9. Median sagittal section through the hypophysis and infundibulum of a larva ten days old. × 60.

FIG. 10. Transverse section of the hypophysis and base of the infundibulum of a larva ten days old. × 190.

FIG. 11. Median sagittal section through the hypophysis and infundibulum of a larva fourteen days old. × 60.

FIG. 12. Median sagittal section through the hypophysis and adjacent parts of a larva about the same age as the preceding. × 190.

FIG. 13. Transverse section of the hypophysis and infundibulum of a larva about fifteen days old. × 60.

FIG. 14. Transverse section through the hypophysis and infundibulum of a larva between twenty-two and twenty-six days old. × 60.

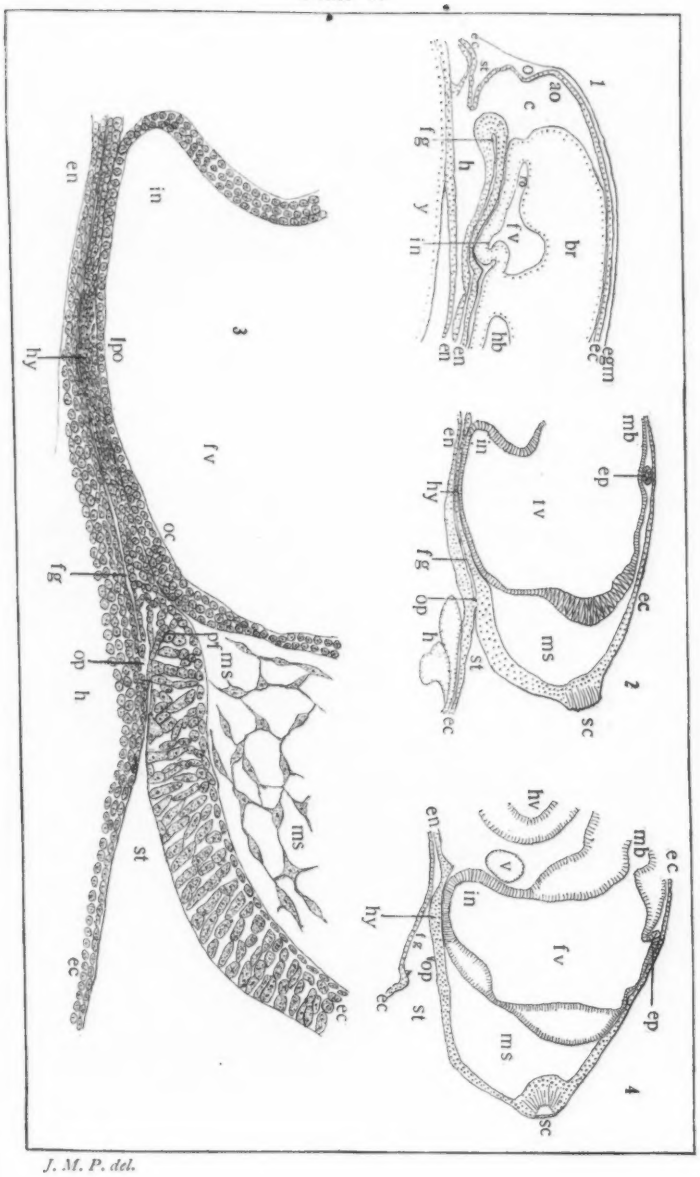
FIG. 15. The hypophysis of the same section more highly magnified. × 215.

FIG. 16. Median sagittal section through the hypophysis and infundibulum of a larva between thirty and thirty-five days old. × 60.

FIG. 17. Transverse section through the hypophysis and infundibulum of a larva of the same age as the preceding. × 60.

FIG. 18. A nearly horizontal section through the hypophysis of a larva about thirty days old. × 105.

Plate I.



J. M. P. del.

Plate II.

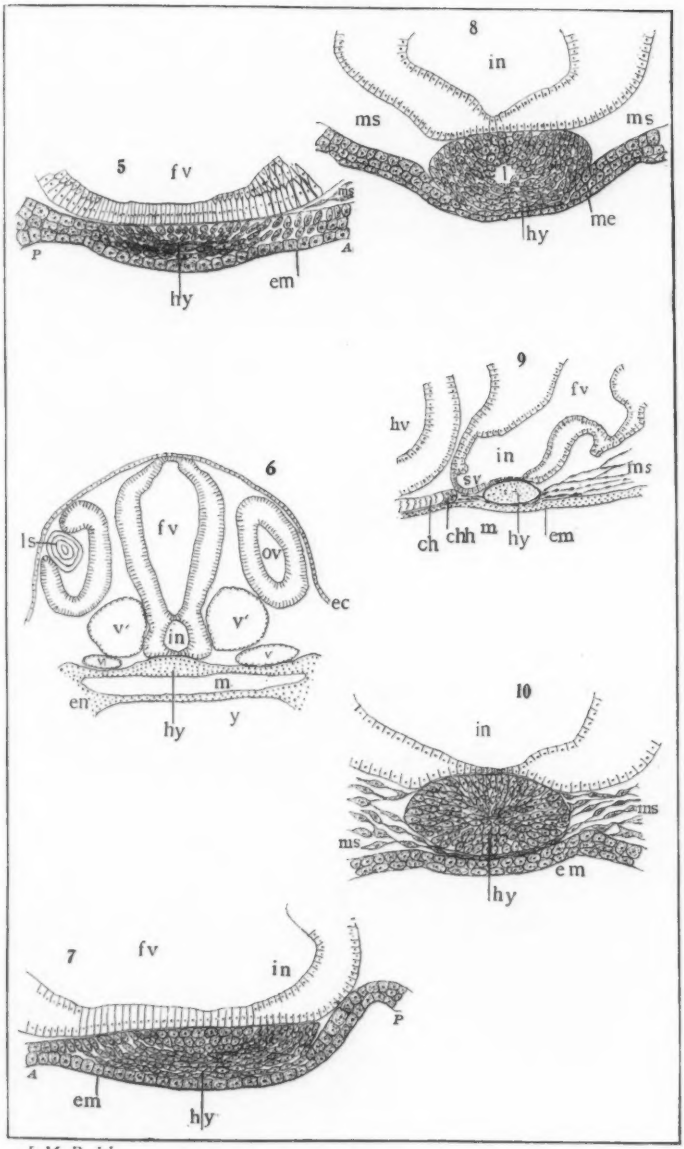


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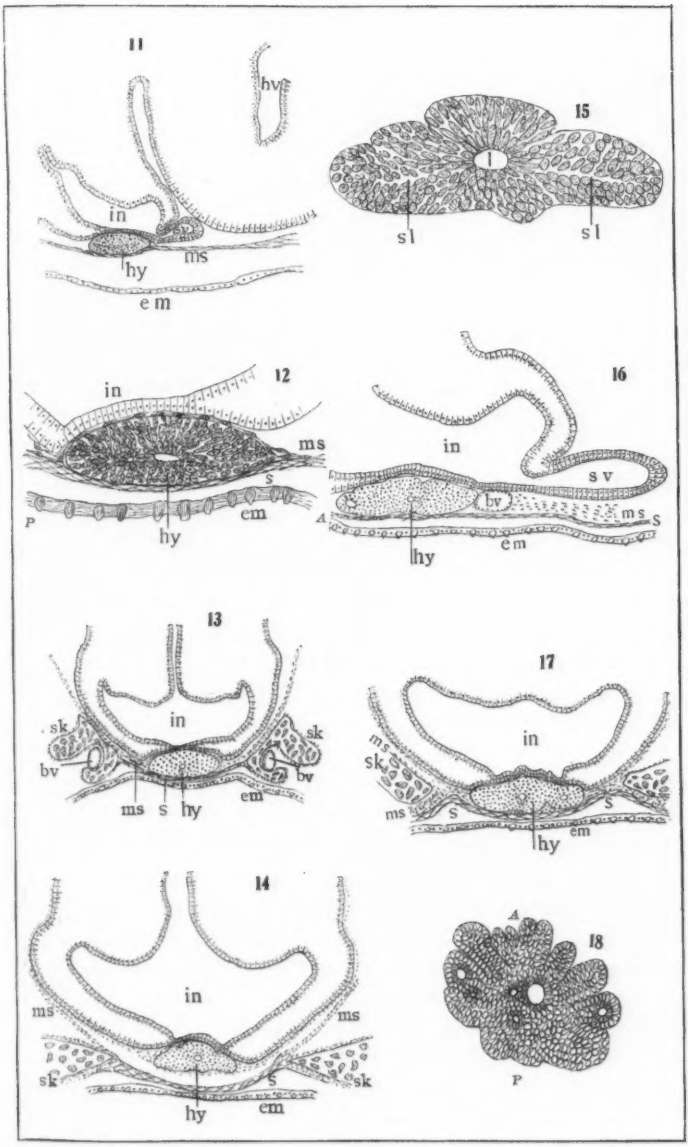
Plate III.



J. M. P. del.

AN EXTRAORDINARY NEW MARITIME FLY.

VERNON L. KELLOGG.

THERE have been established recently two new[1] families of flies, to which I have to add a third. In the case of two of these three new families the members show great divergence from the usual dipterous condition. The three genera of Wandolleck's new family, Stethopathidae, are wingless and are without halteres. The thorax is greatly reduced and the compound eyes are feebly developed. The mouth-parts are of the general sort possessed by the Nematocera, *i.e.*, a short lip-like labium without pseudo-tracheae, a distinct labrum, and a hypopharynx, but no mandibles nor maxillar lobes. Coquillet's new family, the Stenoxenidae, established for a single female fly, presents no such extraordinary characters as the Stethopathidae. "The shape and structure of the head, body, and legs, and the unusual development of the first antennal joint appear to indicate its nearest approach to the genus Ceratopogon of the family Chironomidae; but the venation as well as the general appearance of the insect is very different from anything now located in that family" (Coquillet).

There has come into my hands a number of specimens, 153 in all, of a fly which must prove of unusual interest to zoölogists and entomologists, both because of its peculiar habitat and of its extraordinary structural condition. This new fly can certainly not be ascribed to any known dipterous family; its affinities can only be determined in the most general way. I feel constrained to establish for it a new family, which may be called the Eretmopteridae.

The 153 specimens of the new form, 139 males and 13 females, and 1 female pupa, were collected on Dec. 27, 1898,

[1] Wandolleck, Bruno, "Die Stethopathidae, eine neue Dipteren-Familie," *Zool. Jahrb.* Bd. xi, pp. 412–441, Pls. XXV and XXVI. 1898.

Coquillet, D. W., "A New Dipterous Family Related to the Chironomidae," *Ent. News.* Vol. x, pp. 60 and 61 (figure). March, 1899.

by Mr. J. C. Brown, a student assistant in my laboratory, at Point Lobos, a rocky point on the Pacific Coast near Monterey, California. The flies, of which there were many ("thousands," says Mr. Brown), were resting or running on the surface of the ocean water of tide pools and had a tendency to gather in large numbers in "patches" and in "ball-like masses" on the water. None were seen below the surface, nor were any seen flying. They moved about on the surface of the water very rapidly. In the last week of March, 1899, I visited Point Lobos and searched carefully for the fly, examining the same tide pools on which

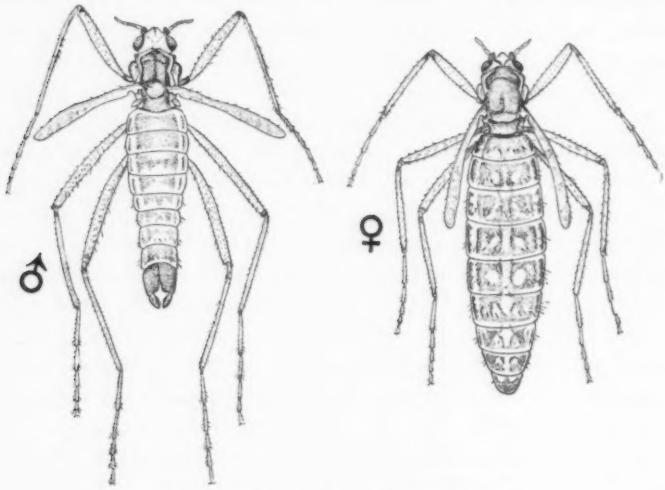


FIG. 1.—*Eretmoptera browni*; male and female.

Mr. Brown found his specimens; but no flies were to be found, nor were there any dipterous larvae or pupae in these pools. Mr. Brown also searched again in July and August without finding more specimens. So we have as yet no knowledge of the eggs and larvae, nor of the course of the life history of the fly.

The new form may be named and described as follows:

Eretmoptera browni nov. gen. et sp.—Male (Fig. 1). Length 2 mm. Head slightly broader than thorax; eyes widely separated, very small, very convex, hairy, and with rather large

facets; ocelli absent; antennae (Fig. 3, *ant.*) short, length 3 mm., 6-segmented, the basal segment wide and globose, the sixth segment longest, the second next, the third and fifth about equal, the fourth shortest, with a few short strong hairs on each segment, and the surface everywhere with a fine stiff pubescence. The mouth-parts are of simple nematocerous type, short, and with distinct labrum-epipharynx, maxillae, hypopharynx, and labium, mandibles absent; labrum-epipharynx (Fig. 2,

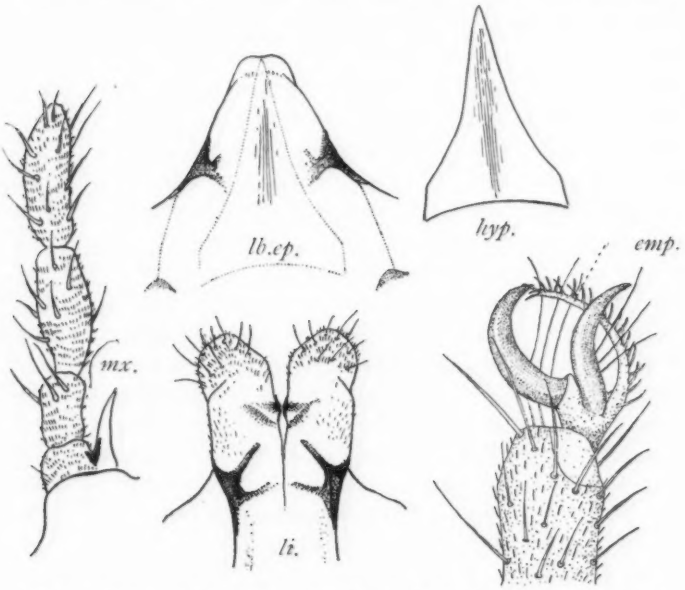


FIG. 2.—*Eretmoptera browni*: *mx.*, maxilla of male; *lb.ep.*, labrum-epipharynx of male; *li.*, labium of male; *hyp.*, hypopharynx of male; *emp.*, empodium of male.

lb.ep.) short, broadly triangular, with obtusely rounded tip; maxillae (Fig. 2, *mx.*) with short, weak, tapering, pointed lobe, and 4-segmented palpi, 3 mm. long; the palpi with last two segments longest and equal, and all the segments provided like the antennae with a few short stray hairs and a fine stiff pubescence; hypopharynx (Fig. 2, *hyp.*) elongate, triangular, as long as the labrum-epipharynx, but narrower and more acute; labium (Fig. 2, *li.*) short, lip-like, with free paraglossae, without pseudo-

tracheae. The face is whitish, with a median longitudinal dark line, and the antennary fossae with dark margins; the basal segment of the antenna is rather dark, the other segments pale. Thorax without bristles, dark above, pale beneath. Legs long and slender, whitish with blackish joints; middle and hind legs longest and equal, front legs only a little shorter; average measurement of middle leg, femur 1 mm., tibia 1 mm., tarsus 1 mm.; tarsus 5-segmented, segment 1 as long as segments 2, 3, and 4 together; segment 5 slightly longer than segment 4; tibiae of all legs with single apical spur; tarsal claws strongly curved, thickened at base, and with a few (three ?) delicate spines on basal half; no pulvilli; empodium (Fig. 2, *emp.*) rather long, curving, filiform, and plumose or pectinate for its whole length. Wings narrow, strap-like, extending only to fourth abdominal segment, length .75 mm., and wholly without veins; whitish, somewhat wrinkled, and finely spinulose. These strange veinless wings are not specially thin or delicate, but, on the contrary, are rather thickened, the costal margin being especially thickened and perhaps folded. The halteres (Fig. 3, *h.*), or the structures which occupy the usual position of halteres, are not of the usual pedicel and knob type common among Diptera, but are minute, lobe-, or scale-like processes, appearing like rudiments of metathoracic wings; like the mesothoracic wings, they are rather thickened and are finely spinulose; they are widest at base and taper to a rounded tip; they average .08 mm. in length. Abdomen of nine segments, tapering gradually posteriorly; mottled gray and blackish above, lighter below, palest laterally; a few scattered, small, wholly inconspicuous hairs, the body appearing glabrous; external genitalia consisting of a pair of large, conspicuous, strong, articulated claspers (Fig. 3, *cl.*), which are covered with a pubescence.

Female (Fig. 1). — Length 2.5 mm., thus being one-fourth longer than the male; this extra length is all in the abdomen, which is markedly larger in every way than the abdomen of the male. The head and thorax are narrower than the robust abdomen, which is sub-cylindrical, tapering only slightly posteriorly. Eyes as in male very small, very widely separated, and hairy. Antennae (Fig. 3, *ant.*) only 4-segmented. Mouth-parts

essentially as in male, with, however, appreciable differences in shape; the labrum-epipharynx is narrower at base, and is more pointed apically; the labium with paraglossae separated farther back and slightly narrower. The reduced wings and halteres like those of male, the wings, length .85 mm., slightly longer. The abdomen consists of nine segments mottled blackish, with conspicuous white sutural spaces, caused by the distention of the abdomen. The external genitalia are inconspicuous. There is a short emarginate dorsal plate with rounded tips and a pair of small lateral processes. There appears to be no extrusible ovipositor.

Pupa of Female. — Among the many specimens collected by Mr. Brown, I find a single female pupa. This specimen throws the only light upon the condition of the immature life of the fly that we yet have. The pupa is of that simple unprotected, unmodified type characteristic of those flies, like the Cecidomyidae and Mycetophilidae, whose pupae are protected by lying enclosed in plant tissue. There are no projecting breathing tubes like those of the aquatic pupae, and it would seem that the pupa was quite unfit for an aquatic life. And yet Mr. Brown took this pupa with the imagines from the surface of a tide pool. There is a puzzle here. The pupa may be described as follows: Length 2.5 mm. (as large as adult female). Immediately recognizable as pupa of the female from the similarity in size, shape, and markings. Abdomen just as in adult both as regards size, shape, color, and markings. The antennae, legs, and wings are folded on the lateral and ventral aspects of the anterior part of the body and extend backwards only to (hardly reaching) the posterior margin of the second abdominal segment. There are no external tracheal gills or elongated spiracles (breathing tubes). There are no bristles nor special clinging organs. The pupa is of a very simple, unmodified, unprotected type.

The "extraordinary" features of the external structure of the fly are the condition of the wings and halteres. The condition of the antennae and the empodium is also unusual. The reduction of the wings and loss of flight are accompanied by a reduction of the halteres, the flight-directing (?) organs. The

halteres being not wholly obsolete, but existing still in rudimentary condition, are especially suggestive in their likeness to rudimentary wings. The general affinities of the fly are shown by the character of the mouth-parts (and pupa) to be with the simpler nematocerous families. The habitat is unusual, but of course not unique. The discovery of the conditions of life of the immature stages may, however, give the matter of habi-

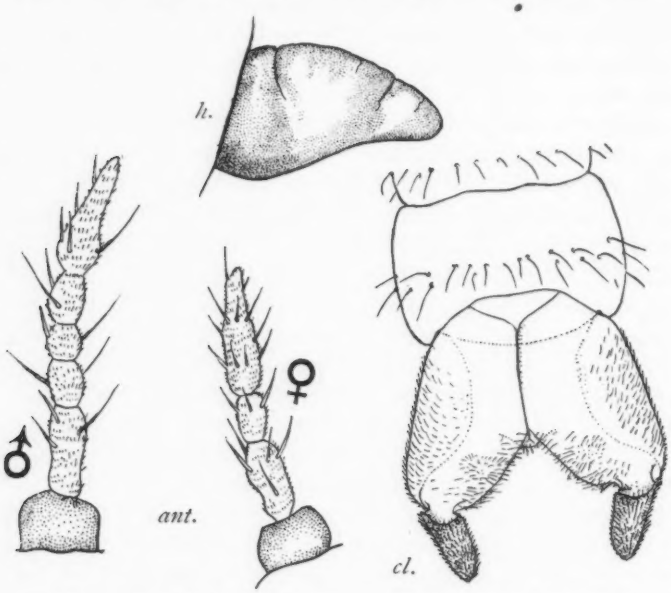


FIG. 3. — *Eretmoptera browni*: *ant.*, antennae of male and female; *h.*, balancer of male; *cl.*, claspers of male.

tat a very great interest. The fact that the imagines are unable to fly is to be remembered in connection with their presence on the tide pools. Are the thickened strap-like reduced wings used in locomotion at all?

Other tide-pool flies are known. Various winged forms are common at the verge of the water and must become accustomed to occasional watery overwhelmings. Wheeler[1] has

[1] Wheeler, W. M., "A Genus of Maritime Dolichopodidae New to America," *Proc. Cal. Acad. Sci.*, 3d series. Vol. i, pp. 145–152, Pl. IV. 1897.

described three species of Dolichopodidae which he found "flitting about in the spray of the breakers among the seaweeds on the rocks below high-water mark." Eaton, and later Verrall,[1] describe certain flies from the Kerguelen Islands which live on the verge of the tide. One of these forms, *Halirytus amphibius*, assigned to the Chironomidae, has 6-segmented antennae and rudimentary wings "reaching to the apex of the first abdominal segment." But it has but 2-segmented palpi, and the halteres are of the usual form. It was found "walking upon the surface of puddles and tide pools." And many of the flies were undoubtedly occasionally submerged at high tide, although none was seen under water.

STANFORD UNIVERSITY, CALIFORNIA.

[1] Verrall, in *Phil. Trans. Royal Soc.* Vol. clxxxvi, p. 247, Pl. XIV, Fig. 6. London, 1879.

ON THE VARIATION IN THE POSITION OF THE STOLON IN AUTOLYTUS.

P. CALVIN MENSCH.

In the investigation of the variation in the position of the region of stolonization in bud-forming syllidians, observations were made upon four of the most abundant forms occurring along the Atlantic coast.

Three of these forms — Autolytus cornutus, Autolytus varians, and Proceraea ornata — may be found in abundance at Woods Holl in the summer months, during which time also the phenomenon of budding is most active. The fourth, Proceraea tardigrada, occurs in almost equal abundance at Beaufort, N. C. Of these A. cornutus, P. ornata, and P. tardigrada are solitary stolon-bearing, and invariably cast off the first stolon before a trace of a second stolon appears, while A. varians belongs to the chain-forming variety and may bear as many as eight stolons in various stages of development attached to the adult individual or so-called parent stock.

The greatest number of variations were observed in the chain-bearing form A. varians. The range in the position of the chain stolon in this species is from segment 19 to 58. The largest percentage of individuals were found to bear the chain on or between segments 30 and 38; a smaller percentage on or between segments 39 and 48, and an equal number between segments 25 and 29; fewer between segments 19 and 24, and a very few between segments 50 and 58.

In 155 individuals examined the following results were tabulated. (The upper numerals indicate the number of the segment to which the chain of stolons is attached; the lower, the number of individuals.)

A	Seg.	30	31	32	33	34	35	36	37	38
	Ind.	16	5	19	4	20	10	5	14	13

B	Seg.	25	26	29	39	40	41	45	48
	Ind.	5	4	9	9	6	4	2	3

C	Seg.	19	21	24	52	58
	Ind.	1	1	2	2	1

From this it will be noted that the position of the chain occurs most frequently on some segment in Table *A*, varying between segments 30 and 38, with a decided preponderance in favor of segments 34, 32, and 30, respectively. In other words, the greatest number of individuals have parent stocks of medium length, with from 30 to 38 segments; and the position of the chain on parent stocks of fewer or more numerous segments than this range occurs less frequently as the number of segments becomes less or greater.

Within the range in which the chain has been found to occur most frequently, it will also be noticed that several segments (31, 33, 36) bear the chain much less often than others, so that the chain-bearing phenomenon would appear to be confined more particularly to certain ones of the segments in this range (30, 32, 34, 37, 38).

In observing the sex of the individuals tabulated, it was noted that by far the greater majority of the specimens with the chain attached to the thirtieth segment or anterior thereto bore female stolons, while those with a great number of segments invariably bore male stolons. In this lot of specimens examined no individuals with female stolons were found with a parent stock of more than 41 segments, while those of 19, 21, and 24 segments all bore female stolons. On the other hand, those of 45 and more segments all bore male chains. Bearing in mind that the first stolon of the series in the chain is formed by the separation of a number of segments from the posterior part of the parent stock, this condition might possibly be due to the fact that the female stolons always consist of a far greater number of segments than do the male, and hence in the process of forming the first stolon leave a more reduced parent stock; while in the process of forming the first male stolon fewer segments would be required, and hence a longer parent stock left back.

In P. ornata and P. tardigrada variations in the position of

the stolon are of comparatively infrequent occurrence. The position of the stolon is very constantly on the thirteenth segment. In more than 200 specimens of P. ornata examined, not more than nine were found in which the stolon was attached to any other than the thirteenth segment; of these, six were found to bear the stolon on the fourteenth segment, while in three it was borne on the twelfth segment. In a single instance I have found the stolon as far forward as the eleventh segment. In all cases the variation was among individuals which bore male stolons.

P. tardigrada shows even less variation than P. ornata. In 55 individuals examined, not a single case of variation was observed. Out of 110 individuals examined, Andrews (*Proc. U. S. Nat. Mus.*, Vol. XIV) obtained three specimens with the bud attached to the fourteenth segment.

Variations in the position of the stolon in the two species of Proceraea would accordingly be confined almost exclusively to occurrences of the position of the stolon either immediately anterior or posterior to a fixed segment, the posterior position being the most frequent form of variation.

In A. cornutus variations in the position of the bud are of still less frequent occurrence. Out of 178 specimens examined at a time when the forms were most abundant, not a single case of variation was observed. I have, however, found specimens in which the stolon was attached to the twelfth segment, but, as compared with Proceraea, such occurrences are very rare.

A further proof of the more constant occurrence of the bud on the thirteenth segment in this species is the fact that in individuals in which mutilation or severance of segments anterior to the region of budding has occurred, in the subsequent regeneration of new segments, the bud will not, as I have observed in Proceraea, develop its head from the tissues of the most anterior regenerated segment, but will first of all regenerate the lost segments of the parent stock, and following these produce the bud. The accompanying figure represents a specimen in which all the segments of the parent stock posterior to the eleventh were amputated and have been replaced by a series of new segments. The bud, instead of taking its origin in the

plane of new tissue formation, *i.e.*, on the twelfth segment, has followed the law of budding in this species and developed its head from the tissues of the fourteenth segment, and thus added two segments to the parent stock. The addition of new tissue to the parent stock, after amputation of one or two of its posterior segments, I have never been able to find in Proceraea, and so far as I have been able to carry my observations, I am convinced that such a condition does not take place, but that instead, wherever new segments are formed following amputation, the head takes its origin from the tissues of the first new segment formed.

9, *10*, *11*, old segments of parent stock; *12'*, *13'*, regenerated segments of parent stock; *a*, stolon with developing head.

From these observations it would appear, therefore, that by far the greatest variation occurs in the chain-bearing form of Autolytus. The great range in the position of the chain, and hence the amount of variation, is modified by a condition to which I have already referred in a previous paper (*Journal of Morphology*, Vol. XVI, No. 2). I have there shown that in this species, in the region in which new segments are being formed (region of proliferation), not all the newly formed segments are pushed back for the formation of new stolons, but that occasionally some of these segments become segments of the parent stock, and thus considerably increase its length. In this way I endeavor to account for the great length (40–58 segments) of the parent stock in the stouter and more developed specimens — a length which I have shown does not exist in the younger and more slender forms. This being the case, the true range of variation would have to be sought in such specimens only which are in the act of forming a first stolon by the separation of the posterior segments of the parent stock. Of such individuals I have found specimens sufficient to give a range from segment 19 to as high as segment 38. Thus while variation here, as identical with the mode of variation in the species investigated, does not present so wide a range as indicated in the tabulation, nevertheless it shows a far greater range and is of much more frequent occurrence in chain-forming

than in the solitary stolon forms. In marked contrast to the variations in A. varians is the constancy in the position of the bud in A. cornutus, where new segments even are formed for the maintenance of a fixed position of budding.

URSINUS COLLEGE, COLLEGEVILLE, PA.,
Nov. 16, 1899.

GORDIACEA FROM THE COPE COLLECTION.

THOS. H. MONTGOMERY, JR.

AT his death Prof. Edward D. Cope left to the Philadelphia Academy of Natural Sciences, among other alcoholic collections, a few specimens of Gordiacea. Among them is a new species, while the others are interesting from the standpoint of geographical distribution.

1. *Gordius aquaticus* (Linn). One ♂ from Haines Falls, Catskills, New York, U. S. A. This specimen is a typical one of this species, and is particularly interesting as coming from such an eastern locality of the United States; previous specimens I have described only from Mexico and California, while all others of the species seen by me from the eastern part of the continent belonged to the following subspecies:

2. *G. aquaticus robustus* (Leidy). Three ♂♂ from Austin, Texas, the first record from this State.

3. *Paragordius varius* (Leidy). One ♂ from the same locality.

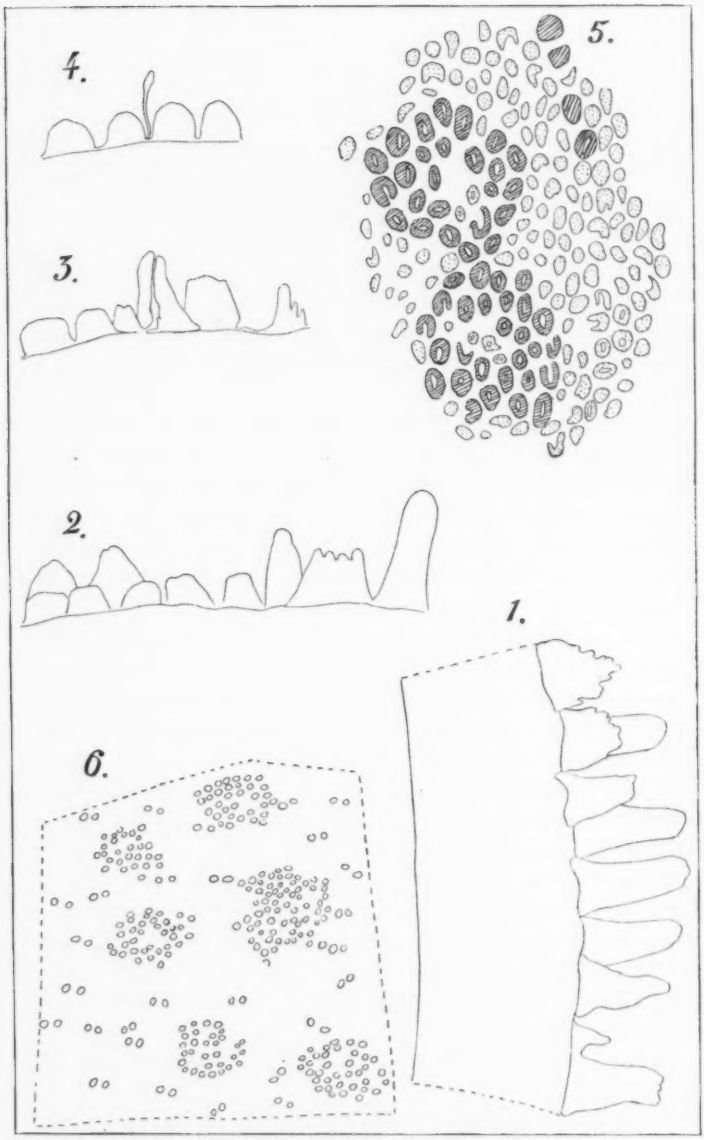
4. *Chordodes occidentalis* (Montg.). One ♂ from Texas, taken from the abdomen of a large grasshopper.

5. *C. Cameranonis*, n. sp. One ♂ from the West Coast, "Mazatlan or Panama." This type is in the collection of the Philadelphia Academy of Natural Sciences.

Form. — Body dorso-ventrally flattened, with slight median grooves. Anterior third of the body gradually tapering to a point, but the extreme end of the head truncated. Posterior end also attenuated, but less so than head; rounded terminally. A post-cloacal ventral groove is in the males of most species of the genus.

Cuticle. — Three main kinds of cuticular processes may be distinguished:

(1) The most abundant are low tubercles, which, on surface view (Fig. 5), appear more or less rounded or oval in outline, less frequently notched at one side, or sickle-shaped. While on



Figs. 1–4, portions of transverse sections of the cuticle, the cuticular processes shown only in outline; in Fig. 1 the outline of the fibrous cuticle is shown. (Zeiss homog. 1 mm. $\frac{1}{12}$, oc. 2.) Fig. 5, surface view of a portion of the cuticle, showing both tubercles and papillae (the latter darker). (Oc. 4, obj. C.) Fig. 6, surface view of the cuticle, seen in water, to show only the arrangement of the groups of papillae, the tubercles not being shown. (Obj. A, oc. 4.)

surface view they appear to be more or less clearly separated from one another, on cross-section they are seen to be connected at their bases. On section (Figs. 2–4) most of them appear of a conical or rounded conical outline, with rounded or flattened smooth summits; but in some the summits are notched or toothed, and this is especially the case with those tubercles found on the margins of the papillar groups next to be described.

(2) Papillae, which on surface view of the cuticle (Fig. 5) appear darker than the tubercles just described, owing to their greater height. On the surface of the cuticle they are arranged in groups of two kinds (Fig. 6, where only these groups of papillae are shown, and none of the tubercles; and Fig. 5, where both tubercles and papillae are shown): (*a*) In larger groups consisting of from about twenty-five to fifty papillae (usually about forty) each; and (*b*) in pairs, the pairs being much more numerous than the larger groups. The line joining the two papillae of a pair lies in the transverse axis of the body, and not infrequently two or three pairs of papillae may lie in such close juxtaposition as to form transverse rows of four or six papillae each. In the larger groups of papillae the center of each group is occupied by papillae of less height than those on the periphery, or is devoid of papillae. These papillae may be readily recognized on surface view, in addition to their dark coloring, by the clearer central portion, which is often narrow and slit-like (Fig. 5).

Transverse sections of the cuticle (Figs. 1–4) show these papillae in two forms. First, there are long, comparatively slender, finger-shaped papillae, with smooth outlines and rounded summits; some of these attain a height nearly equal to the transverse diameter of the underlying fibrous portion of the cuticle. And, secondly, there are papillae of greater diameter at the base, but less height, which lie among the former kind, and may be distinguished by the irregular notching and tuberculation of their summits and sides. The clear central portion of these papillae seen on surface view is shown on cross-section to be a clear axial core running the whole length of the papilla and representing possibly a pore canal.

On none of these papillae have I been able to find terminal

hairs, such as are so frequently found in the larger papillae of many species of the genus; no hairs were to be seen on the cuticle on surface view examined in water and in balsam, nor yet on cross-sections examined with the one-twelfth immersion lens of Zeiss.

(3) Hyaline, club-shaped, delicate processes scattered sparingly over the cuticle (Fig. 4).

Color. — Black, head rufous-brown.

Dimensions. — Length, 425 mm.; greatest diameter, 2 mm. The structure of the genital organs showed this specimen to be sexually mature.

Comparisons. — This appears to be a well-marked species. In the arrangement of the papillae it bears some resemblance to *C. festae* Camerano, but differs from the latter in having no fine hairs ("corti e fini prolungamenti trasparenti") on the summits of the papillae and also in having no large, transparent, recurved hooks on the surface of the cuticle (*cf.* Camerano, "Monografia dei Gordii," *Accad. Real. Sci. di Torino*, 1897, p. 386, Taf. III, Fig. 38).

It is a pleasure to me to name this species in honor of Prof. Lorenzo Camerano, of Turin, who has given such an able systematic monograph of the group.

Biological School,
University of Pennsylvania, Philadelphia,
December 11, 1899.

A PRELIMINARY ACCOUNT OF THE SPERMATOGENESIS OF BATRACHOSEPS ATTENUATUS, POLYMORPHOUS SPERMATOGONIA, AUXOCYTES AND SPERMATOCYTES.

GUSTAV EISEN, PH.D.

INTRODUCTORY.

THIS paper is a preliminary report of my investigations on the spermatogenesis of *Batrachoseps attenuatus*. The memoir will be published in the *Journal of Morphology*, Vol. XVII, No. 1. As, however, some considerable time must elapse before the paper can be published, it has been deemed proper to publish this short extract covering a few of the more important points.

Batrachoseps is adult in the months of June and July, and is at this time difficult to find, as it is then estivating deep below the surface. The testes were fixed by my iridium-chloride method and sectioned in paraffin. The stain used was the iron haematoxylin, according to Benda, and after-staining with congo. The sections were studied with Zeiss Apochromate, 2 mm. Ap. l. 40. The light used was the incandescent gas-light passing through the achromatic filter described lately in the *Zeitschrift f. wiss. Microscopie*, Bd. XIV, p. 444.

The figures illustrating this paper are entirely diagrammatic. No special effort has been made to insert the correct number of chromioles in the chromosomes; and the author's want of skill in preparing this kind of drawing will account for many other discrepancies.

CONSTITUENTS OF THE CELL.

The following general division of the structures of the cells of the testes is proposed: Cytosome, Caryosome, and Archo-

some. This division is in accordance with the one proposed in my paper on the plasmocytes in the blood of *Batrachoseps*.[1]

The cytosome comprises all that part of the cell situated exterior to the nucleus except the archosome. The cytosome contains the following distinct structures: cytoplasm proper, plasmosphere, hyalosphere, granosphere, metaplasmic secretions, cytoplasmic membrane, and cell wall. None of these

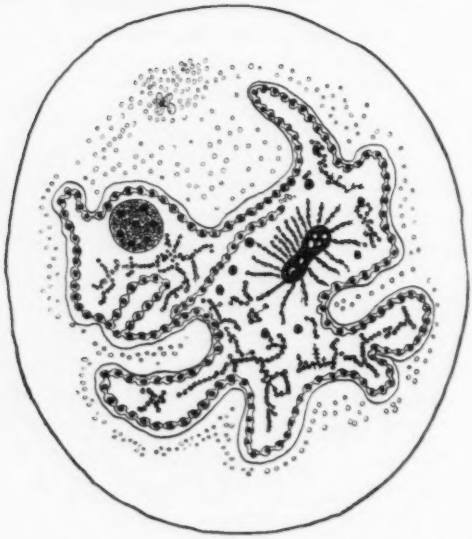


FIG. 1. — A polymorphous spermatogonium in the "perfect resting stage." The form of the nucleus allows the most perfect metabolism. Numerous chromioles are connected by a thread of chromoplasma. A network of linosomes is partially indicated, the individual granules being connected by Linopodia. A large chromoplast with endochromatic granules. Eight parachromatic granules. A single archosome in the cytoplasm, the latter only partially indicated by small open circles. A single large linoplast, with seven endonucleolar granules.

structures are in any way intimately connected with the archosome. The three spheres mentioned above surround each other, like three concentric shells, at the time when the cell is in partial resting stage; but at a later stage, or as soon as the prophase is entered, these spheres break up and scatter in the cytoplasm proper. Each one of the spheres constitutes an independent structure, and they are not developed one from the other.

[1] *Proc. Cal. Acad. Sci.*, 3d Ser., Zoöl. Vol. i, No. 1.

The plasmosphere is the outermost sphere; the granosphere is the innermost one. The plasmosphere is the first one to break up into minor parts. These arrange themselves in the equatorial of the cell and serve as material for the mantle fibers

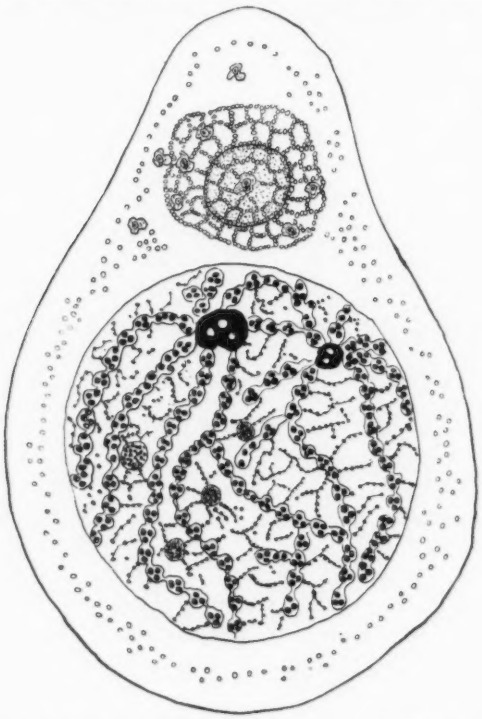


FIG. 2. — Auxocyte in the "imperfect resting stage," showing the formation of leaders consisting of round chromioles surrounded by a film of chromoplasm. The leaders start from two chromoplasts of unequal size, both containing endochromatic granules. The leaders are connected by a linosomic network. Four linoplasts. In the cytoplasm are seen the two spheres, the inner one, the granosphere, containing the archosome. There are eight accessory archosomes, some in the plasmosphere, others in the cytoplasm. The two spheres are of a foam-like structure. The cytoplasm is only partially indicated.

and for the new cell wall which is formed when the two daughter-cells separate.

The granosphere remains longer, but when it breaks up it furnishes material for the fibers of the central spindle. It also constitutes the main dwelling place of the archosome.

The archosome, or centrosomal structures, consists of three distinct parts, situated one interior to the other. These three parts constitute one single organized and individualized body — the archosome. The most interior part is the centriole; this centriole is surrounded by a thin layer or zone — the somosphere. The somosphere is surrounded by a generally non-staining zone — the centrosphere. There are one or more centrioles.

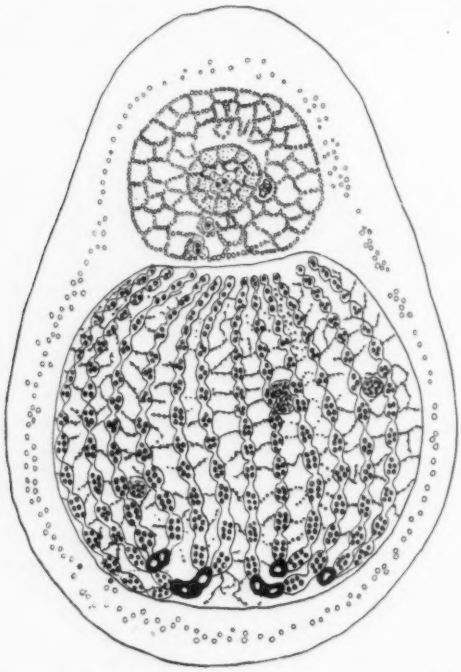


Fig. 3. — An auxocyte in the "bouquet stage." There are twelve leaders starting from five chromoplasts. The leaders consist of chromomeres containing chromioles suspended in a film of chromoplasm. The spheres are of a foam-like structure. There are three accessory archosomes and one archosome with two centrioles. The open space between the inner granosphere and the outer plasmosphere represents the hyalosphere. The cytoplasm is only partially indicated.

Both the somosphere and the centrosphere are amoeboid, especially the centrosphere. The latter constitutes the organ of locomotion of the archosome. There are besides the archosome several accessory archosomes. The function of the

archosome is to conduct the development and evolution of the fibers of the mitotic figures. The function of the accessory archosomes is to conduct the formation of the contractile fibers and perhaps furnish material for their construction. They also conduct the "fiber cones."

The location of the archosome is variable; it is sometimes situated in the granosphere, but is at other times found outside

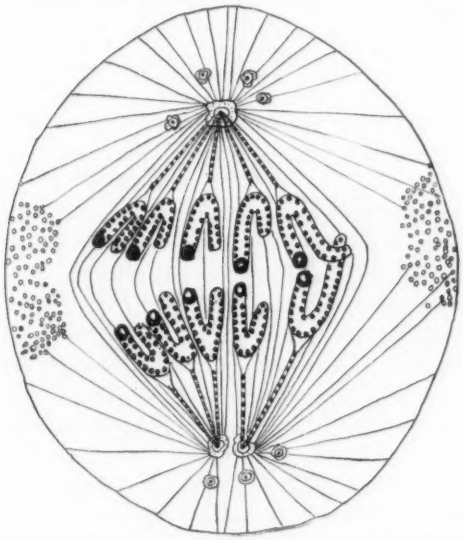


FIG. 4.—An auxocyte in the beginning of the anaphase. Only a few of the chromosomes are indicated. At each pole there are one and two archosomes and three and four accessory archosomes. The chromosomes contain chromioles suspended in chromoplasm. At the apex of each chromosome there is seen a chromoplast with endochromatic granules. To the right and left in the cell are seen agglomerations of plasmosphere indicating the position of the new cell wall, which is to separate the two daughter-cells. The chromosomes are seen to be connected with the chromiole by contractile fibers, the latter consisting of granules enclosed in a common sheath. The spindle fibers as well as the polar fibers start from the centrosphere.

of it. The accessory archosomes are of the same structure as the archosome, and one of the former may assume the function of the latter.

The accessory archosomes, if too numerous, are expelled from the cell, and then become paracellular bodies. Similarly, parts of the spheres are also expelled from the cell.

The nucleus contains the following more or less distinct

parts: chromioles, chromomeres, chromosomes, chromoplasts, linoplasts, linin, chromoplasm, endochromatic granules, and parachromatic granules.

Chromioles. — These are the most minute of the visible organized and individualized primary structures of the nucleus, and are the most important constituents of the chromosomes, probably being the carriers of heredity. They appear as minute globules, staining darker than the other parts of the nucleus except the chromoplasts. The chromioles are of a certain size and number in every species of nucleus and in every perfect chromosome. They are, as a rule, arranged in a regular manner in the chromosomes and in the chromomeres. During the absolute resting stage of the cell the chromioles are situated free in the nucleus, connected only by tiny filaments of linin and chromoplasm; while during the mitotic stages they are grouped into chromomeres, and these again into chromosomes. With absolute resting stage is indicated only absolute rest from "mitotic work." During this stage active metabolism is carried on.

There are thirty-six chromioles in every perfect chromosome, and these are divided among six chromomeres, each chromomere containing six chromioles. The chromioles are surrounded by a connective, apparently homogenous substance — the chromoplasm. The chromoplasm thus constitutes the greatest bulk of the chromosome.

The chromomeres are small aggregations of chromioles from three to six in number, according to the stage of development of the nucleus. The chromosomes in the polymorphous nuclei are twenty-four in number, but in the other testes cells there are only twelve. Each perfect chromosome contains six chromomeres.

In the resting stage of the polymorphous spermatogonia we find in the nucleus one or more large dark-staining bodies — the chromoplasts (net-knots). These chromoplasts are particular and most important organs of the nucleus. Their function is to attract the chromioles and to arrange them first into leaders, and later, through certain changes of the leaders, into chromosomes. The chromoplasts finally divide up into as many parts as there are chromosomes, one part adhering to each chromo-

some through its entire existence. The chromoplasts are characterized by one or more highly refractive endochromatic granules, which probably serve as nourishment for the chromi-

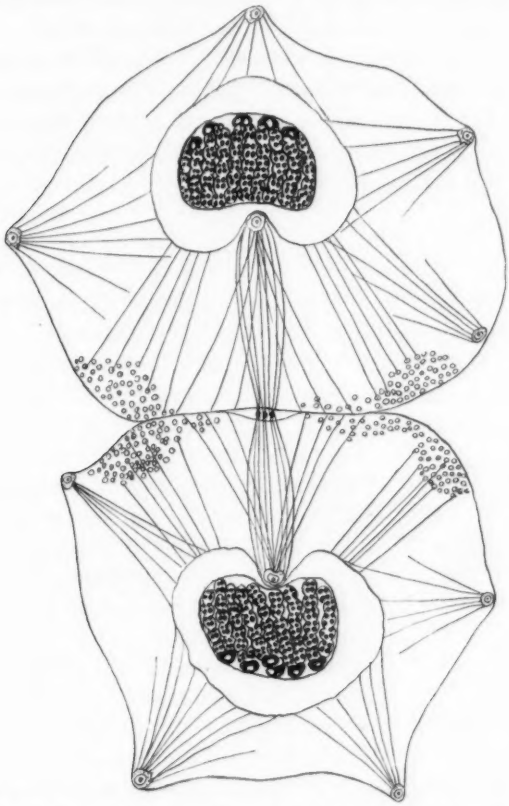


FIG. 5.—Two daughter-cells of an auxocyte connected by a spindle bridge. There are eight accessory archosomes at the apex of as many fiber cones. Two archosomes are connected by a central spindle. In the latter is seen a mid-body consisting of three condensation granules. The chromosomes are being regenerated, and the chromoplasts appear at the angle of the chromosomes instead of at the apex, as in the last cell stage. In one nucleus are seen five, in the other six chromoplasts with endochromatic granules. Between the true nuclear membrane and the false membrane is an open space caused by the false membrane being pulled away by the fiber cones.

oles. The chromoplasts serve as landmarks by which the position of the chromosomes can be ascertained with great accuracy.

The linin consists of minute granules — linosomes — arranged in a more or less regular network, which latter at certain times supports the elements of the chromosomes. The true nucleoli or linoplasts are principally agglomerations of linosomes, and serve as storage reservoirs for the linin network.

The nuclear membrane is formed apparently from linin and not from cytoplasm proper. During the anaphase a false nuclear membrane is formed from cytoplasm proper, but this membrane is again dissolved as soon as the object for which it is formed is accomplished.

SPINDLES AND SPINDLE FIBERS.

The following varieties may be segregated: mantle fibers, polar fibers, central spindle fibers, contractile fibers, retractile fibers, and fiber cones. All these fibers originate only in connection with an archosome. The contractile fibers alone are directly connected with the centriole of the archosome. All the other fibers and rays emanate from the outer margin of the centrosphere of the archosome, but do not penetrate into this sphere, and accordingly are not connected with the centrioles or the somosphere. The material for the mantle fibers is furnished from the granules and the secretions of the plasmosphere; while the material for the central spindle is furnished by the granules and secretions of the granosphere.

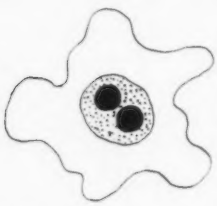


FIG. 6. — An archosome consisting of an outer centrosphere, an inner somosphere, with two centrioles.

The contractile fibers are those which connect the chromosomes with the archosome. They are from the beginning of a different structure from any of the other fibers, being beaded and highly contractile. Their structure strongly recalls that of muscle fiber.

The fiber cones are particular structures, so far not met with in any other cells. They consist of bundles of fibers held together at one point by an accessory archosome, while the distal ends of the fibers are attached to the false nuclear membrane

formed around the nucleus at the time of the anaphase. The archosome moves away and carries with it the fibers, which pull away the false nuclear membrane, thus causing a vacuole to form around the nucleus. The object of all this is probably to enable the nucleus to develop without the interference of surrounding structures. These fiber cones are frequently very numerous, as many as seventeen having been counted in a single cell. They are of large size and cause the cell membrane to be pushed out.[1]

The spindle bridge, which connects two or more cells, consists of the remnant of the central spindle. As the spindle bridge exists only in cells which commence the same phase of mitosis at the same time, it is probable that the purpose of the spindle bridge is to time or regulate the commencement of this mitosis. The mid-body of the spindle bridge serves probably as a storage reservoir for the cytoplasm of the spindle bridge.

Varieties of Cells.

The testes of *Batrachoseps* contain four distinct varieties of cells, as follows : polymorphous spermatogonia, auxocytes, spermatocytes, and spermatids. These originate one from the other in the order mentioned above. Of these varieties there are one or more generations. They are characterized as follows :

Polymorphous Spermatogonia. — These possess a perfect resting stage in which the nucleus is polymorphous as regards form, being greatly indentated and folded during the perfect resting stage. The nucleus during this stage contains neither chromosomes nor chromomeres, the chromioles being scattered about and not connected with the chromoplasts. These cells give rise to several generations of cells of the same nature, with the exception that there is no perfect resting stage like the one in the mother-cell, and that consequently the nucleus is not folded, but perfectly even, round, or oblong. The mitosis of the poly-

[1] Fiber cones of similar appearance, but of a different nature, have been described by botanists from the pollen cells of higher plants. These cones, however, do not possess archosomes.

morphous spermatogonia and their offspring is through twenty-four chromosomes and somatic division. The last generation of these cells gives rise to the auxocytes.

The auxocytes are characterized as follows: They possess a bouquet stage; they are the first cells with twelve chromosomes; their mitosis is heterotypic and by equation; they pos-

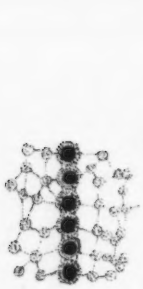


FIG. 7.

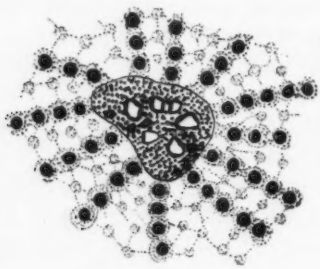


FIG. 8.

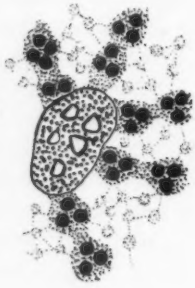


FIG. 9.

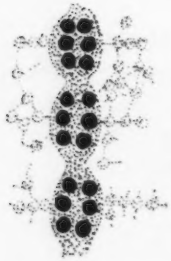


FIG. 10.

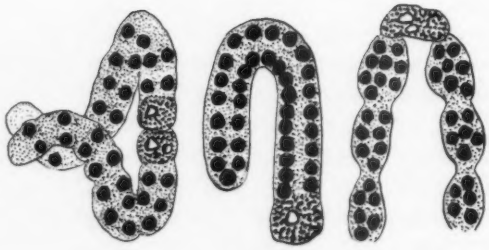


FIG. 11. FIG. 12. FIG. 13.

FIGS. 7–13 represent a broken series of leaders illustrating the formation of the leader and the chromosome.

FIG. 7. — Isolated row of chromioles surrounded by chromoplasm and suspended in a network of linosomes.

FIG. 8. — Chromoplast with twelve leaders of chromioles. From the imperfect resting stage of the polymorphous spermatogonium.

FIG. 9. — Chromoplast with five leaders. Each leader is made up of chromomeres, and each chromomere consists of three or more chromioles surrounded by chromoplasm. A network of linosomes between the chromomeres.

FIG. 10. — Three chromomeres, each with six chromioles surrounded by a chromoplasm and suspended in a network of linosomes.

FIG. 11. — A pretzel chromosome containing chromioles and two chromoplasts with endochromatic granules.

FIG. 12. — A chromosome from the metaphase. It contains thirty-six chromioles and a terminal chromoplast with an endochromatic granule.

FIG. 13. — Part of a chromosome from the spermatocyte.

sess no perfect resting stage, the chromioles being arranged into leaders and always connected with the chromoplasts; there is but one generation; the daughter-cells are the spermatocytes; and they have numerous fiber cones at the end of the anaphase.

The spermatocytes are characterized as follows: They have numerous fiber cones in the beginning of the mitosis, homotypic mitosis with twelve chromosomes and with equation division; no bouquet stage and no perfect resting stage; but one generation; the daughter-cells are the spermatids which give rise to the spermatozoa through direct development and growth; and the chromosomes are *V*-shaped.

The Mitosis.

The mitosis is the result of two distinct and separate processes which, for the greater part, run parallel and independent of each other, but which meet at certain nodes in order to

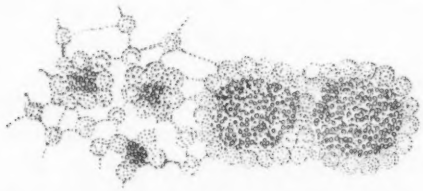


Fig. 14.—A diagrammatic representation of the structure of the granosphere. The dotted globules are cytoplasmic granules, and between them are seen metaplasmic secretions represented by small open rings. The globules are connected by Linopodia, and form a foam structure, partly a network.

accomplish certain objects jointly. These processes are the chromosomic process and the radiosomic process.

The radiosomic process is presided over by the archosome and the accessory archosomes, and consists in the development and evolution of the various fibers and the central spindle, in the evolution of the spheres, and the dissolution of the nuclear membrane. To this process belong also the development and dissolution of the false nuclear membrane and the reabsorption of the fibers.

The chromosomic process is presided over by the chromo-

plasts, and consists in the development and evolution of the leaders out of chromoplasm and the chromioles, the formation of the latter into chromomeres and chromosomes, and the multiplication of the chromioles and their proper distribution in the chromosomes. The two processes coöperate in the separation and equation of the chromosomes, which coöperation commences with the dissolution of the nuclear membrane. To the chromosomic process belongs also the movement of the chromoplasts in the umbrella-shaped and confluent nucleus at the end of the anaphase. With this process the archosomes have nothing to do, as it is accomplished before the nuclear membrane is dissolved by the mantle fibers.

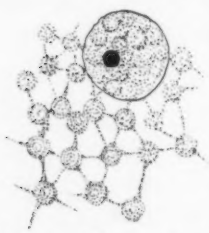


FIG. 15. — A diagrammatic representation of the structure of linosomes and the linoplast. The individual linin granules are connected by means of Linopodia. The linoplast contains linosomes as well as an endonucleolar body.

The radiosomic process commences with the dispersion of the spheres. The plasmosphere is dispersed first, and its granules are arranged in the equatorial of the cell, there to furnish material for the new cell walls. The central spindle fibers are then formed out of material furnished by the granosphere, which is in this way entirely used up. The nuclear membrane is dissolved by the mantle fibers and not by the central spindle fibers. The contractile fibers are formed after the central spindle fibers have reached considerable size.

The chromosomic process begins with the formation of leaders out of chromioles and chromoplasm. The chromioles aggregate into chromomeres, and, later on, a certain number of these form chromosomes. Their formation is shortly as follows: The leaders to the number of twelve are connected with the chromoplasts, and by contraction and a certain arrangement assume the bouquet stage. The leaders then split lengthwise, the two forks being held together by a fragment of the chromoplasts. The chromoplast divides into as many parts as there are to be chromosomes, but each part is always attached to a leader. Next, the two halves of the leader spread apart and twist around each other and thus form a pretzel-shaped chromosome. By this time the nuclear membrane is dissolved and

the pretzel-shaped chromosomes are placed on the central spindle, where they are taken hold of by the contractile fibers, which attach themselves to the prongs halfway between the

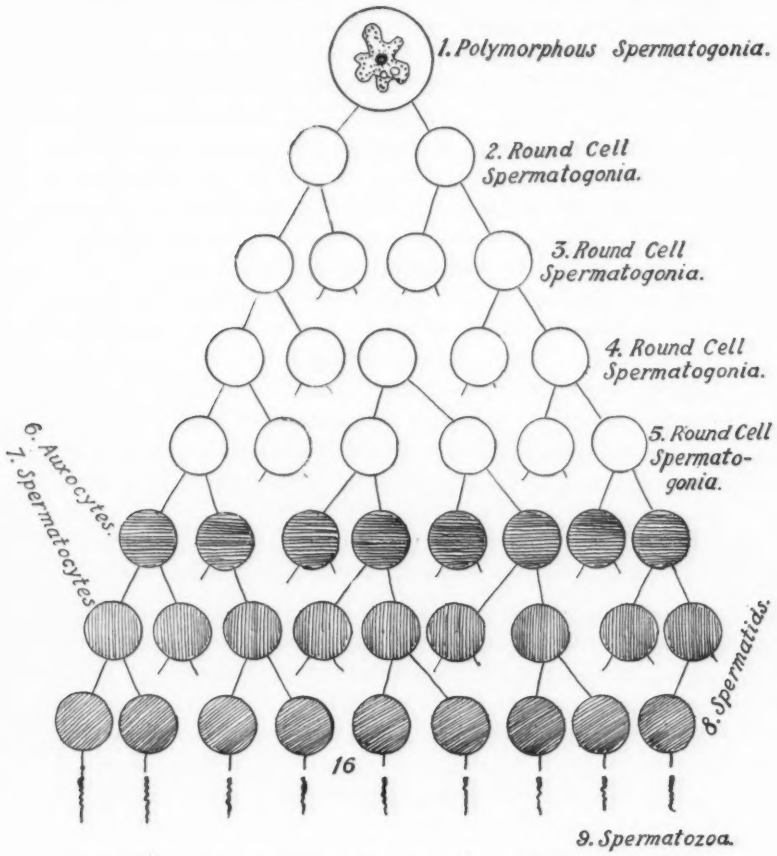


FIG. 16. — Diagram of the cell generations in *Batrachoseps* testes; 1, polymorphous spermatogonia; 2 to 5, four generations of round cell spermatogonia; 6, auxocytes; 7, spermatocytes; 8, spermatids; 9, spermatozoa.

chromoplasts and the free end. Each half is then pulled away and the chromosome is formed by an equation and not by a reduction. In the new chromosome the fragment of chromo-

plast is attached to one of the ends. This process is the one that takes place in the auxocytes.

The next step is the formation of a confluent umbrella stage or ring-like nucleus. The object of this form is to allow the chromoplasts to change their place. When the nucleus is reorganized in the spermatocyte the chromoplasts are found to be situated not at the end of each chromosome, but at the angle of the fork. This change of position could not take place except through the medium of an umbrella-shaped nucleus. During this stage the chromioles are also doubled. The nucleus now passes through a stage of growth which is facilitated through the large vacuole which is formed around the nucleus with the aid of the fiber cones and the accessory archosomes.

In the spermatocyte the central spindle is frequently formed from two opposite fiber cones left over from the last mitosis. The chromosomes of the spermatocytes are *V*-shaped before mitosis. They are divided longitudinally in the way usual in the homotypic mitosis, and by equation, not by reduction. During the prophases of the radiosomic mitosis the superfluous archosomes are expelled from the cell and remain for some time as paracellular bodies between the cells.

PERMANENCY AND NATURE OF THE CELL STRUCTURES.

The cytosome proper contains no permanent structures of any kind. The plasmosphere, hyalosphere, granosphere, the various kinds of fibers, as well as the central spindle, are all ephemeral structures which are developed by rearrangement of preëxisting granula, and which again disperse when their function is over. The granula contained in the cytosome is at least of four different kinds, and everything points to the conclusion that one kind of granula is never converted into any other kind. In other words, the granula of the granosphere is not evolved from the granula of the plasmosphere, etc., but both are independent and individualized primary structures as compared with the secondary ones of spheres and fibers. For the principal granula of the cell the following terminology is proposed : cytosomes, plasmosomes, hyalosomes, somosomes, granosomes, and linosomes, the latter being of nuclear origin.

If we turn to the nucleus, we find similarly that the chromomeres, the chromosomes, and the leaders are also ephemeral and secondary structures which form and disperse, the chromioles alone being the permanent individualities of the chromosomic structures. The nucleus then contains the following permanent granula: linosomes, the chromoplasmic granula, the chromioles, and the granula composing the chromoplasts. The permanent structures of the cell are the centrioles, the chromioles, and the chromoplasts. As regards the primary parts of these last-mentioned structures we are yet in doubt, but there is every reason to believe that these structures are of a highly complicated nature.

Similarly the fibrillar and alveolar structures of the protoplasm are only secondary, ephemeral, and temporary. With proper optical means we see that the alveole, as well as the reticulum, is built up of granules. These granules adhere to each other by means of minute projections or arms, for which I have proposed the name of Linopodia. The ultimate visible structure of the protoplasm is thus a granule, capable of projecting and retracting Linopodia.

For a fuller explanation and demonstration of these facts I must refer to the larger paper now in the hands of the publisher of the *Journal of Morphology*.

Biological Laboratory,
California Academy of Sciences,
San Francisco, California.

ERRATUM.

In No. 1, p. 1, 13th line, last word, read *ganglion* in place of "*gland.*"